

Appendix D3

**Summary Minutes of Independent Scientific Peer Review Panel Meeting on April 28-29,
2009**

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Summary Minutes

Independent Scientific Peer Review Panel Meeting

Assessing the Allergic Contact Dermatitis Potential of Chemicals and Products: Evaluation of the Updated Validation Status of New Versions and Applications of the Murine Local Lymph Node Assay (LLNA)

William H. Natcher Conference Center

National Institutes of Health

Bethesda, MD

April 28 - 29, 2009

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
Nathalie Alépée, Ph.D.	Scientific Coordinator on Alternatives Methods in Life Science, L'Oréal Research and Development, Aulnay sous Bois, France
Anne Marie Api, Ph.D.	Vice President, Human Health Sciences, Research Institute for Fragrance Materials, Woodcliff Lake, NJ
Nancy Flournoy, M.S., Ph.D.	Professor and Chair, Dept. of Mathematics and Statistics, University of Missouri – Columbia, Columbia, MO
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
Michael Olson, Ph.D.	Director of Occupational Toxicology, Corporate Environment Health and Safety, GlaxoSmithKline, Research Triangle Park, NC

Peer Review Panel Members:

Raymond Pieters, Ph.D. ⁴⁰	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
Jonathan Richmond, MB ChB, FRCSEd	Head, Animals Scientific Procedures Division, Home Office, London, U.K.
Peter Theran, V.M.D.	Massachusetts Society for the Prevention of Cruelty to Animals, Novato, CA
Stephen Ullrich, Ph.D.	Dallas/Ft. Worth Living Legends Professor and Professor of Immunology, Postgraduate School of Biomedical Science, University of Texas M.D. Anderson Cancer Center, Houston, TX
Michael Woolhiser, Ph.D.	Science and Technology Leader – Toxicology and Environmental Research and Consulting, The Dow Chemical Company, Midland, MI
Takahiko Yoshida, M.D., Ph.D.	Professor, Dept. of Health Science, Asahikawa Medical College, Hokkaido, Japan

ICCVAM and ICCVAM Immunotoxicity Working Group Members:

Paul Brown, Ph.D.	FDA, Center for Drug Evaluation and Research, Silver Spring, MD
Masih Hashim, Ph.D.	EPA, Office of Pesticide Programs, Washington, DC
Ying Huang, Ph.D.	FDA, Center for Biologics Evaluation and Research, Silver Spring, MD
Abigail Jacobs, Ph.D. (IWG Co-Chair)	FDA, Center for Drug Evaluation and Research, Silver Spring, MD
Jodie Kulpa-Eddy, D.V.M.	USDA, Animal and Plant Health Inspection Service, Riverdale, MD
Elizabeth Margosches, Ph.D.	EPA, Office of Pollution Prevention and Toxics, Washington, DC
Joanna Matheson, Ph.D. (IWG Co-Chair)	CPSC, Bethesda, MD

⁴⁰ Dr. Pieters was unable to attend the public meeting on April 28-29, 2009. However, he was involved in the review of the revised draft background review documents and the revised draft LLNA applicability domain Addendum.

ICCVAM and ICCVAM Immunotoxicity Working Group Members:

Deborah McCall	EPA, Office of Pesticide Programs, Washington, DC
Tim McMahon, Ph.D.	EPA, Office of Pesticide Programs, Washington, DC
John Redden, M.S.	EPA, Office of Pesticide Programs, Washington, DC
R. Adm. William Stokes, D.V.M., DACLAM	NIEHS, Research Triangle Park, NC
Ron Ward, Ph.D.	EPA, Office of Pollution Prevention and Toxics, Washington, DC
Marilyn Wind, Ph.D. (ICCVAM Chair)	CPSC, Bethesda, MD

Invited Experts:

George DeGeorge, Ph.D., DABT	MB Research Labs, Spinnerstown, PA
Kenji Idehara, Ph.D.	Daicel Chemical Industries, Ltd., Hyogo, Japan
Masahiro Takeyoshi, Ph.D.	Chemicals Evaluation and Research Institute, Saitama, Japan

JaCVAM Observer:

Hajime Kojima, Ph.D.	National Institute of Health Sciences, Tokyo, Japan
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Public Attendees:

Joan Chapdelaine, Ph.D.	Calvert Laboratories, Inc., Olyphant, PA
Merrill Tisdell	Syngenta Crop Protection Inc., Greensboro, NC
Gary Wnorowski, M.B.A, L.A.T.	Eurofins Product Safety Labs

NICEATM:

R. Adm. William Stokes, D.V.M., DACLAM	Director
Debbie McCarley	Special Assistant to the Director
Contract Support Staff – Integrated Laboratory Systems, Inc. (ILS)	
David Allen, Ph.D.	Eleni Salicru, Ph.D.
Thomas Burns, M.S.	Frank Stack

NICEATM:

Linda Litchfield

Judy Strickland, Ph.D., DABT

Greg Moyer, M.B.A.

Abbreviations:

CPSC = U.S. Consumer Product Safety Commission

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 = Immunotoxicity Working Group

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

NIEHS = National Institute of Environmental Health Sciences

NIOSH = National Institute of Occupational Safety and Health

USDA = U.S. Department of Agriculture

Tuesday, April 28, 2009

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, and members of the public to also introduce themselves. Dr. Luster stated that there would be opportunity for public comments during each of the four murine 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 comments from one individual would be welcomed during each commenting period, repeating the same comments at each comment period would be inappropriate.

Welcome from the ICCVAM Chair

Dr. Marilyn Wind, U.S. Consumer Product Safety Commission (CPSC) and Chair of ICCVAM, welcomed everyone to the National Institutes of Health and to the Panel meeting. Dr. Wind thanked the ICCVAM IWG and NICEATM staff for their efforts in preparing the draft documents being reviewed and for arranging the logistics of the meeting. Dr. Wind thanked the Panel members for dedicating their time, effort, and expertise to this review 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 an NIH Special Emphasis Panel and was being held in accordance with applicable U.S. Federal Advisory Committee Act regulations. As such, Dr. Stokes indicated that he would be serving as the Designated Federal Official for this public meeting. He reminded the Panel that they signed a conflict of interest (COI) statement during the Panel selection process, in which they identified any potential real or perceived COI. He read the COI statement and then Dr. Luster asked that panelists again declare any potential direct or indirect COI and to recuse themselves from discussion and voting on any aspect of the meeting where there might be a conflict.

Dr. Michael Woolhiser declared a COI regarding the Panel's review of the LLNA Applicability Domain, because The Dow Chemical Company, Dr. Woolhiser's employer, submitted much of the data that were being considered. He indicated that he would recuse himself from the Panel's evaluation of the applicability domain, but would remain available to answer any questions that the Panel might have about the test substances or the data.

Overview of the ICCVAM Test Method Evaluation Process

Dr. Stokes began by thanking the 15 Panel scientists from six different countries (Czech Republic, France, Japan, The Netherlands, United Kingdom, and the United States) for their significant commitment of time and effort preparing for and attending the meeting. He explained that the purpose of the Panel was to conduct an independent scientific peer review of the information provided on a series of proposed new versions of the LLNA and proposed expanded applications of the assay. The Panel is then asked to comment on the extent that the available information supports the draft ICCVAM recommendations. Dr. Stokes indicated that the original LLNA peer review panel in 1998 considered the LLNA a valid substitute for the guinea pig-based test in most but not all testing

situations. He noted 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 2007,⁴¹ 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 the results of validation studies for proposed test methods to assess 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 2000,⁴² including the purpose and duties of ICCVAM. He noted that one of ICCVAM's primary 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 processes, and helps to facilitate not only the acceptance of scientifically valid alternative test methods, but also encourages internationally harmonized recommendations on the usefulness and limitations of alternative test methods.

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 and determines whether the test method should move forward into a formal evaluation. If so, 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 available information and develops draft test method recommendations on the proposed usefulness and limitations of the test methods, test method protocol, performance standards, and future optimization/validation studies. The draft 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 draft 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 developing final recommendations. These final recommendations are forwarded to the ICCVAM member agencies for their consideration and possible incorporation into relevant testing guidelines. Agencies have 180 days to respond to the ICCVAM recommendations.

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.

⁴¹ http://iccvam.niehs.nih.gov/methods/immunotox/llnadocs/CPSC_LLNA_nom.pdf

⁴² http://iccvam.niehs.nih.gov/docs/about_docs/PL106545.pdf

ICCVAM Charges to the Panel

Dr. Stokes reviewed the charges to the Panel: (1) review the draft BRDs and the draft Addendum to the traditional⁴³ LLNA 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 appropriately addressed for the proposed revised or modified versions of the LLNA; and (3) comment on the extent to which the ICCVAM draft test method recommendations including the proposed usefulness and limitations, standardized test method protocols, performance standards, and additional studies are supported by the information provided in the draft BRDs and draft Addendum.

Overview of the Agenda

Dr. Luster then reviewed the agenda and the order of presentations. He stated that for each review topic, the test method developer would present an overview of the test method protocol, followed by a presentation by NICEATM staff summarizing each revised draft BRD, and lastly a member of the IWG would present the draft ICCVAM recommendations. Following presentations, the Panel Evaluation Group Leader for the topic under consideration would present the group's draft recommendations, followed by Panel discussion. Public comments would then be presented, followed by the opportunity for additional Panel discussion in consideration of the public comments. The Panel would then vote to accept the Panel consensus, with any minority opinions being so noted with the rationale provided for the minority opinion.

Current Regulatory Testing Requirements and Hazard Classification Schemes for Allergic Contact Dermatitis (ACD) and the Traditional LLNA Procedure

Dr. Matheson presented an overview of ACD and relevant regulatory requirements. She briefly discussed the ICCVAM final recommendations for the LLNA Performance Standards, the updated ICCVAM LLNA test method protocol, and the reduced LLNA (rLLNA), all of which were reviewed by the Panel at their meeting in March 2008.

The Panel questioned who was responsible for conducting the future studies referred to in the revised draft ICCVAM test method recommendations. Dr. Stokes replied that these recommendations are provided for consideration by the stakeholder community. Those organizations with appropriate resources can use this information to guide their research, development, and validation activities.

A question arose from the Panel as to why pooled data (as opposed to individual animal data) are collected for the LLNA.

Dr. Matheson replied that, pooled data are often collected since OECD Test Guideline 429 allows the use of a minimum of four animals per treatment group when collecting pooled data, but requires a minimum of five animals per treatment group when collecting individual animal data. Legislation in some countries, and many Animal Care and Use Committees, require that the test method to be used is the one requiring the fewest animals. Dr. Matheson also noted that the ICCVAM LLNA test method protocol has recently been revised to allow the use of a minimum of four animals per treatment group when collecting individual animal data, so there is now no reason not to collect individual animal data. At the Panel meeting in March 2008, the Panel stated that all future LLNA studies should require that lymph nodes be collected from individual animals instead of pooling them

⁴³ 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.

with other animals in a treatment group since individual animal response data allows for identification of technical problems and outlier animals within a dose group.⁴⁴

A question arose as to whether the U.S. Environmental Protection Agency (EPA) prefers LLNA or guinea pig data for submission. Dr. Matheson ceded the floor to Ms. Debbie McCall of EPA Office of Pesticide Programs, who was in attendance. Ms. McCall said that EPA prefers LLNA data, but will accept either guinea pig maximization test (GPMT) or Buehler test (BT) data.

Overview of the Revised Draft LLNA: DA Test Method Procedure BRD and Revised Draft ICCVAM Test Method Recommendations

The first test method reviewed was the LLNA: DA test method. This test method measures the ATP content of lymph node cells by the luciferin/luciferase method, as an index of lymphocyte proliferation, after exposure to a test substance.

Dr. Kenji Idehara of Daicel Chemical Industries, Ltd., Japan (the test method developer) presented a synopsis of the test method to the Panel.

A Panelist asked about the half-life of ATP in the lymph node cells after the mouse is sacrificed. Dr. Idehara replied that the ATP concentration declines 20 to 30% in an hour, with a half-life of about 2 to 2.5 hours. The assay time from animal sacrifice to complete measurement of ATP content for each individual animal is maintained as similar as possible, within approximately 30 min. He also said that the time between sacrifice and ATP assay is not a problem when collecting individual animal data, if the time between the excision of the lymph nodes, the preparation of the cell suspensions, and the measurement of the ATP concentrations is kept relatively constant between animals.

A Panelist asked if the lymph node samples were randomized before the ATP assays were conducted. Dr. Idehara replied that the samples were not randomized.

On behalf of NICEATM, Dr. Salicru presented an overview of the revised draft LLNA: DA BRD to the Panel.

A question arose about NICEATM's use of different decision criteria for the accuracy analysis, and the reproducibility analyses in the revised draft BRD. Dr. Salicru noted that a decision criterion of $SI \geq 2.5$ was used for the reproducibility analyses because it was found to be the optimal decision criterion for identifying sensitizers (i.e., it resulted in a 0% false positive rate).

Dr. Wind presented the revised draft ICCVAM test method recommendations for the LLNA: DA test method to the Panel. She noted that ICCVAM favored the multiple decision criteria to eliminate any false positives or false negatives. A Panelist commented that, as more data are accumulated using the test method, false positives and false negatives might appear.

A Panelist asked, if the true stimulation index (SI) value for a compound was 2.0, if that compound would be classified as a sensitizer or a nonsensitizer. Dr. Wind replied that, as described in the revised draft ICCVAM recommendations, other information would be necessary to definitively answer that question.

Dr. Kojima presented the results of the Japanese Society for Alternatives to Animal Experiments (JSAAE) interlaboratory validation studies of the LLNA: DA and the LLNA: BrdU-ELISA test methods to the Panel. In the presentation, he noted that the JaCVAM Regulatory Acceptance Board has examined the results of the studies for both test methods and accepted the LLNA: DA as a replacement for the traditional LLNA. The JaCVAM Regulatory Acceptance Board has requested additional data for the LLNA: BrdU-ELISA.

⁴⁴ http://iccvam.niehs.nih.gov/docs/immunotox_docs/LLNAPRPRpt2008.pdf

Panel Evaluation:

Dr. Woolhiser presented the draft position developed by Evaluation Group B, which was charged with primary review of the LLNA: DA test method. The Panel agreed that the available data and test method performance support the use of the LLNA: DA to identify substances as potential skin sensitizers and nonsensitizers, with certain limitations. They concurred with ICCVAM's proposal that, based on the current validation database, the multiple SI decision criteria should be used to identify sensitizers and nonsensitizers (i.e., $SI \geq 2.5$ for sensitizers, $SI \leq 1.7$ for nonsensitizers). The Panel also noted that the limitation of these test methods when using the proposed multiple decision criteria is the indeterminate classification of substances that fall in the range of SI values for which a classification is uncertain (i.e., $1.7 < SI < 2.5$). The Panel recommended that when such results are obtained, users should carefully interpret the results using an integrated decision strategy in conjunction with all other available information (e.g., dose response and quantitative structure-activity relationship [QSAR] information, peptide-binding activity, molecular weight, results from related chemicals, other testing data) to determine if there is adequate information for an accurate sensitization hazard classification or if additional testing is necessary. The Panel emphasized that, from an animal welfare perspective, retesting should not be undertaken until all other available information is evaluated, and a determination is made that such testing is required to fill a data gap. The Panel also recommended that more detailed guidance be developed for regulatory agencies on how the multiple decision criteria could be used in practice.

Subsequent Panel discussions focused on ICCVAM's recommendation to use multiple decision criteria to identify sensitizers and nonsensitizers. In general, the Panel preferred the multiple decision criteria to a single decision criterion for identifying sensitizers and nonsensitizers. A Panelist recommended that graphs showing the maximum SI obtained with the modified test method (the LLNA: DA, in this case) plotted against the maximum SI obtained with the traditional LLNA, for each test substance, be included in the final BRD. This was a general recommendation for both test methods that use multiple decision criteria (i.e., the LLNA: DA and LLNA: BrdU-ELISA). It was also pointed out that, as more data are accumulated for these test methods, the cut-off SI values for sensitizers and nonsensitizers would likely change.

Bootstrapping analysis was mentioned as a means to provide some measure of variability of the chosen cut-off values. It was also mentioned that the tables in Section 7.0 of the revised draft BRD provide no measurement of variation for the data. It was suggested that all of these tables include treatment means, standard deviations, and the mean squares, so that F-values can be calculated for between and among laboratory means. However, the Panel agreed that, while this information would be useful for inclusion in the final BRD, it would not impact the Panel's overall conclusions about the test method.

Some discussion followed about variations in the LLNA: DA test method protocol from the updated ICCVAM-recommended traditional LLNA test method protocol (i.e., sodium lauryl sulfate pretreatment prior to test substance application and an additional test substance application on day 7). The Panel agreed that despite these variations, the LLNA: DA was still mechanistically and functionally similar to the traditional LLNA.

Public Comments:

At the conclusion of the Panel discussion, Dr. Luster called for public comments. None were presented.

Panel Conclusions and Recommendations:

Dr. Luster asked if the Panel was in agreement with the conclusions in the draft Panel Report as reflected in the updated Evaluation Group presentation as modified during the discussions. The Panel approved unanimously.

Applicability Domain of the LLNA and Revised Draft ICCVAM Test Method Recommendations

NICEATM provided an overview of the revised draft Addendum on the LLNA applicability domain. Subsequent to the 2008 Panel consideration of this topic, new data were obtained for pesticide formulations, dyes, essential oils, and substances tested in aqueous solution, but none were obtained for metals. Since the Panel previously considered the use of the term *mixtures* too broad, data were separately evaluated by product subgroups in the revised draft Addendum, and they were identified in general terms as pesticide formulations and other products. Dr. Wind presented the revised draft ICCVAM test method recommendations for the LLNA applicability domain to the Panel.

Subsequent to Dr. Wind's presentation, Dr. Luster asked Ms. McCall of EPA to clarify EPA's position on the use of LLNA data for pesticide formulations. Ms. McCall replied that EPA accepted positive or negative LLNA data on single substance technical grade additives. Between 2003 and 2007, EPA received few LLNA studies on pesticide formulations. Positive LLNA results were accepted, but for negative results, EPA required a confirmatory test. The majority of sensitization data submitted to EPA for pesticide formulations are from the guinea pig BT. There are limited human data available on pesticides due to the ethics limitations for conducting human studies, and applicants provide all of EPA's data.

A Panelist commented that the GPMT is more sensitive than the BT; he said that, in his experience, the GPMT showed roughly 60% positive results versus 20% positive results for the BT, for the same group of formulations. He said that the LLNA is more concordant with the GPMT than it is with the BT. He said that the GPMT is the preferred test in Europe. The Panel agreed that this should be reflected in the comparisons of LLNA and guinea pig results.

Panel Evaluation:

Dr. Olson presented the draft position developed by Evaluation Group A, which was charged with primary review of the LLNA applicability domain, to the Panel. While the Panel agreed that there were too few data in the revised draft Addendum for some of the test substance classes (e.g., dyes, essential oils) to make a firm statement about concordance of the LLNA with other test methods for these classes, the Panel stated that any material should be suitable for testing in the LLNA unless there is a biologically-based rationale for exclusion, such as unique physicochemical properties that might affect their ability to interact with immune processes. The Panel therefore agreed that the LLNA should be considered appropriate for testing pesticide formulations and other products, unless there is a biologically-based rationale for exclusion.

The Panel also concurred that, while studies done with BALB/c mice should not be excluded from the evaluations in the revised draft Addendum, CBA should remain the preferred strain for the updated ICCVAM-recommended LLNA test method protocol, and that the use of any other strain, or of male rather than female mice, should be justified by the investigator.

The Panel did not agree that Pluronic L92 should be added to the list of preferred vehicles for the LLNA, but it did agree that studies done with Pluronic L92 should not be excluded from the evaluations in the revised draft Addendum.

While the concordance of LLNA results for essential oils was properly compared with human results, the Panel noted that the revised draft Addendum neglected to consider information that showed LLNA results were more concordant with human results when the major component was $\geq 70\%$, compared to the concordance for the essential oil itself. The Panel also commented that the term *natural complex substances* was more appropriate for these types of substances than *essential oils*, because this is the terminology used for the Registration, Evaluation, Authorisation and Restriction of Chemical substances program now in force in the European Union (EU).

In reference to the data for the medical device eluates in the revised draft Addendum, the Panel commented that ISO Standard 1099 requires the chemical analysis of such materials before skin sensitization testing is undertaken, and therefore agreed that the data provided were of little use for evaluating the performance of the LLNA for testing these types of substances.

Public Comments:

At the conclusion of the Panel discussion, Dr. Luster called for public comments.

Mr. Gary Wnorowski, Eurofins Product Safety Labs

Mr. Gary Wnorowski said he had registered to make a public comment, but that Ms. McCall of EPA had already addressed his question by her answer to Dr. Luster's question regarding acceptability of pesticide formulation data.

Panel Conclusions and Recommendations:

Dr. Luster asked if the Panel was in agreement with the conclusions in the draft Panel Report as reflected in the updated presentation. The Panel approved unanimously.

Adjournment

At the conclusion of the discussion on the applicability domain, Dr. Luster adjourned the Panel for the day at 5:30 p.m., to reconvene at 8:30 a.m. on Wednesday, April 29, 2009.

Wednesday, April 29, 2009

Overview of the Draft LLNA: BrdU-ELISA Test Method Revised Draft BRD and Revised Draft ICCVAM Test Method Recommendations

Dr. Luster called for Panel consideration of the LLNA: BrdU-ELISA test method. This test method measures bromodeoxyuridine (BrdU), a thymidine analog, instead of radioactive thymidine, incorporated into the DNA of proliferating lymphocytes, via an enzyme-linked immunosorbent assay (ELISA).

Dr. Masahiro Takeyoshi of Chemicals Evaluation and Research Institute, Japan (the test method developer) presented a synopsis of the test method to the Panel.

On behalf of NICEATM, Dr. Strickland presented an overview of the revised draft ICCVAM LLNA: BrdU-ELISA BRD to the Panel.

A Panelist asked why ICCVAM proposes an SI value of 2.0 as the cutoff value for a sensitizer instead of a value of 2.5, since the data indicated that no false positives would result if either value were used. Dr. Strickland replied that the value of 2.0 was chosen because this was the lowest value that resulted in a 0% false positive rate, thus minimizing the range of uncertainty.

Dr. Jacobs presented the revised draft ICCVAM test method recommendations for the LLNA: BrdU-ELISA test method to the Panel.

Panel Evaluation:

Dr. Ullrich presented the draft position developed by Evaluation Group B, which was charged with primary review of the LLNA: BrdU-ELISA test method, to the Panel.

The Panel agreed that the LLNA: BrdU-ELISA test method was mechanistically and functionally similar to the traditional LLNA, and the ICCVAM LLNA Performance Standards could be used to evaluate it. The Panel also concurred that the available data and test method performance support the use of the LLNA: BrdU-ELISA to identify substances as potential skin sensitizers and nonsensitizers, with certain limitations. They agreed with ICCVAM's proposal that, based on the current validation database, the multiple SI decision criteria should be used to identify sensitizers and nonsensitizers

(i.e., $SI \geq 2.0$ for sensitizers, $SI > 1.3$ for nonsensitizers). The Panel also noted that the limitation of these test methods when using the proposed multiple decision criteria is the indeterminate classification of substances that fall in the range of SI values for which a classification is uncertain (i.e., $2.0 > SI \geq 1.3$). The Panel recommended that when such results are obtained, users should carefully interpret the results in an integrated decision strategy in conjunction with all other available information (e.g., dose-response and QSAR information, peptide-binding activity, molecular weight, results from related chemicals, other testing data) to determine if there is adequate information for an accurate sensitization hazard classification or if additional testing is necessary. The Panel emphasized that, from an animal welfare perspective, retesting should not be undertaken until all other available information is evaluated, and a determination is made that such testing is required to fill a data gap. The Panel also recommended that more detailed guidance be developed for regulatory agencies on how the multiple decision criteria could be used in practice.

Subsequent Panel discussions focused on ICCVAM's recommendation to use multiple decision criteria to identify sensitizers and nonsensitizers. In general, the Panel preferred the multiple decision criteria to a single decision criterion for identifying sensitizers and nonsensitizers. The Panel agreed that all of the comments for the LLNA: DA test method regarding the graphs and tables in the revised draft BRD, and the provision of measures of variation for interlaboratory reproducibility data, apply to the BrdU-ELISA also.

A Panelist commented that the use of interpolation for determining EC_t values presupposed a monotonic increase in SI values and that isotonic regression might be more appropriate in cases in which a monotonic increase does not occur. More Panel discussion occurred regarding the practical usefulness of the multiple decision criteria. It was agreed that the term *integrated assessment* was more appropriate than *weight-of-evidence* to describe the approach taken to classify substances that fell into the uncertainty range.

The Panel discussed when it was appropriate to rely on hypothesis testing (as opposed to decision criteria based on a cutoff SI value) to classify substances. The Panel commented that, in some cases, statistical significance might not indicate a biological effect. The Panel agreed with the language regarding hypothesis testing in the current ICCVAM LLNA Performance Standards (Appendix A - Section 3.0).

Public Comments:

At the conclusion of the Panel discussion, Dr. Luster called for public comments.

Dr. George De George, MB Research Labs

Dr. De George raised the following points:

- The data evaluated for the 1999 ICCVAM evaluation of the LLNA were statistically analyzed.
- As a result of that analysis, the optimum SI cutoff for a sensitizer was determined as 3.16.
- The Panel for the 1999 evaluation chose 3.0 as the SI cutoff to provide an added level of confidence.
- Routine statistical analysis of LLNA data to classify test substances was not recommended in the 1999 evaluation. In Dr. DeGeorge's opinion, the best reason to collect individual animal data was so that, in the future, studies could be done to determine an optimum method for hypothesis testing of LLNA data.
- Newer variant LLNA tests should be subjected to the same level (and not held to a higher level) of requirements for validation as the traditional LLNA.

Panel Conclusions and Recommendations:

At the conclusion of the public comments, Dr. Luster asked if the Panel was in agreement with the conclusions in the draft Panel Report as reflected in the updated presentation. The Panel approved unanimously.

Overview of the Revised Draft LLNA: BrdU-FC Test Method BRD and Revised Draft ICCVAM Test Method Recommendations

Dr. Luster called for Panel consideration of the LLNA: BrdU-FC test method. This test method measures bromodeoxyuridine (BrdU), a thymidine analog, instead of radioactive thymidine, incorporated into the DNA of proliferating lymphocytes, via flow cytometric analysis. The test method also allows for the measurement of immunophenotypic markers in the lymphocyte population, ostensibly aiding in discrimination between irritants and sensitizers.

Dr. George DeGeorge of MB Research Labs, Spinnerstown, PA (the test method developer) presented a synopsis of the test method to the Panel. In addition to a brief description of the test method protocol, Dr. DeGeorge made the following points:

- The test method protocol was based on the ICCVAM-recommended LLNA test method protocol, using $SI \geq 3.0$ as the decision criterion for a sensitizer.
- Test substances were chosen to include those tested in the traditional LLNA.
- Guinea pig data and human results are considered less reliable.
- The LLNA: BrdU-FC uses lower doses of test substances than the traditional LLNA to avoid irritating concentrations.
- The LLNA: BrdU-FC makes correct calls for some substances for which the traditional LLNA does not.
- All of the data generated by MB Research Labs using the LLNA: BrdU-FC are available for review at the laboratory (although not all data are available electronically).
- MB Research Labs is currently attempting to find other laboratories interested in participating in an interlaboratory validation study.

Following Dr. De George's presentation, a Panelist asked the following questions:

- Does MB Research Labs conduct LLNA: BrdU-FC studies according to GLP? Dr. De George said yes.
- What is the treatment group size? Dr. DeGeorge responded that five animals per treatment group were used.
- Can measurement of ear swelling be added to any LLNA variant test method as an additional endpoint? Dr. DeGeorge replied that it could, and that it could help resolve which doses to test.

On behalf of NICEATM, Dr. Allen presented a summary of the revised draft LLNA: BrdU-FC BRD to the Panel. At the conclusion of Dr. Allen's presentation, Dr. DeGeorge pointed out that an in-house flow cytometer and trained operators weren't necessary to conduct the test method, because the lymphocytes were fixed as part of the test method protocol, and the flow cytometry analysis could be outsourced.

Dr. Jacobs then presented the revised draft ICCVAM test method recommendations for the LLNA: BrdU-FC test method to the Panel.

Panel Evaluation:

Dr. Richmond presented the draft position developed by Evaluation Group B, which was charged with primary review of the LLNA: BrdU-FC test method, to the Panel.

The Panel agreed that the LLNA: BrdU-FC test method was mechanistically and functionally similar to the traditional LLNA, and the ICCVAM LLNA Performance Standards could be used to evaluate it. The Panel also concurred that the database of more than 45 representative test substances yielded adequate accuracy based on results from one laboratory, and that intralaboratory reproducibility also had been adequately demonstrated. However, the Panel agreed with the ICCVAM proposal to defer a formal recommendation on the validity of the LLNA: BrdU-FC until an independent audit of all data supporting the analysis has been conducted and until transferability has been demonstrated in an interlaboratory validation study. The Panel recommended that ICCVAM should work with NICEATM to support and facilitate the independent audit and interlaboratory validation study. The Panel recommended that upon completion of these tasks and determination of satisfactory data quality, power, and interlaboratory reproducibility, that the LLNA: BrdU-FC could be considered to have adequate validation and performance to support its consideration for regulatory use.

Much Panel discussion about the necessary statistical power of the test method occurred. Power is defined as the probability that the test method would determine that a test group showing a positive result is different from the negative control (i.e., that a sensitizer would be detected as such). Data presented to the Panel during their 2008 evaluation indicated that the test method would require nine animals per treatment group to achieve 95% power; the power with five animals per group was estimated at 80% in that evaluation. The Panel agreed that, before an interlaboratory validation study was begun, it should be verified that the LLNA: BrdU-FC test method has power at least equal to that of the traditional LLNA using five animals per treatment group.

Public Comments:

At the conclusion of the Panel discussion, Dr. Luster called for public comments.

Dr. George De George, MB Research Labs

Dr. De George raised the following points:

- Power calculations on a subset of the data are not as reliable as accuracy statistics calculated from the entire dataset for 45 chemicals.
- Power calculations are a new requirement for validation, and not contained in the ICCVAM LLNA Performance standards.
- It was Dr. De George's opinion that it would be difficult, if not impossible, to get three qualified testing laboratories to participate in an interlaboratory validation study.

Panel Conclusions and Recommendations:

Subsequent to the public comments, the Panel commented that the flow cytometric analysis for samples from all three laboratories in an interlaboratory study could be done at MB Research Labs. Power calculations could be done by NICEATM on the most recent data generated by the LLNA: BrdU-FC test method.

The Panel decided to make a nomination to ICCVAM, with high priority, that NICEATM organize and supervise an interlaboratory validation study for the LLNA: BrdU-FC test method.

Dr. Luster asked if the Panel was in agreement with the conclusions in the draft Panel Report. The Panel approved unanimously.

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, the Evaluation Group Chairs, and the experts on the test methods, who presented them to the Panel.

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 test method for the benefit of the Panel. Dr. Stokes concluded by saying he looked forward to further working with the Panel members to complete their Panel report.

Adjournment

Dr. Luster adjourned the Panel at 11:30 a.m., concluding the meeting.