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BALLOT VOTE SHEET

Date: December 21, 2011

TO : The Commission
Todd A. Stevenson, Secretary

FROM : Cheryl A. Falvey, General Counsel
Kenneth R. Hinson, Executive Director

SUBJECT : Staff Response to the ICCVAM Recommendations on Revisions to the Murine Local Lymph Node Assay

BALLOT VOTE due: December 28, 2011

The attached memorandum from the Health Sciences Directorate summarizes the recommendations of the Interagency Coordinating Committee on the Validation of Alternative Methods ("ICCVAM") regarding the Murine Local Lymph Node Assay's ("LLNA") ability to categorize the potency of chemicals causing allergic contact dermatitis. Staff recommends that the Commission accept the ICCVAM recommendations and instruct staff to so inform ICCVAM by letter.

Please indicate your vote.

I. Accept the ICCVAM recommendations and instruct staff to so inform ICCVAM by letter.

_____ _____
Signature Date

II. Reject the ICCVAM recommendations and instruct staff to so inform ICCVAM by letter.

_____ _____
Signature Date

III. Take other action (Please specify).

_____
_____
_____
_____

_____ _____
(Signature) (Date

Attachment - *Staff Response to the ICCVAM Recommendations on the Usefulness and Limitations of the Murine Local Lymph Node Assay for Potency Categorization of Chemicals Causing Allergic Contact Dermatitis*, memorandum from Joanna Matheson, Ph.D., Directorate for Health Sciences, to the Commission, December 2011.

UNITED STATES
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Memorandum

Date: December 16, 2011

TO : The Commission
Todd A. Stevenson, Secretary

THROUGH: Kenneth R. Hinson, Executive Director
Cheryl A. Falvey, General Counsel
Robert J. Howell, Deputy Executive Director for Safety Operations

FROM : DeWane Ray, Assistant Executive Director
Office of Hazard Identification and Reduction
Joanna Matheson, Ph.D., Toxicologist
Directorate for Health Sciences

SUBJECT : Staff Response to the ICCVAM Recommendations on the Usefulness and Limitations of the Murine Local Lymph Node Assay for Potency Categorization of Chemicals Causing Allergic Contact Dermatitis

In early 2007, CPSC sent a nomination to the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) requesting review of the validation status of new versions and applications of the Murine Local Lymph Node Assay (LLNA), including the use of the LLNA for determining skin sensitization potency categories. In the intervening years, ICCVAM has released multiple recommendations on these nominated activities to committee member agencies. On March 9, 2010, the Commission voted unanimously to approve three ICCVAM recommendations regarding the LLNA including: (1) updates to the test method protocol; (2) establishment of performance standards; and (3) a modified form of the assay, the reduced Local Lymph Node Assay (rLLNA). On January 26, 2011, the Commission voted unanimously to approve three ICCVAM recommendations regarding the LLNA (including two nonradioactive versions of the assay): (1) the Bromodeoxyuridine Enzyme-linked Immunosorbent Assay (BrdU-ELISA); (2) the Daicel Chemical Industries version (LLNA:DA); and (3) an update on the LLNA's applicability domain, particularly its effectiveness in testing pesticide formulations, metals, and substances in aqueous solutions. The recommendations, on the ability of the LLNA to categorize the potency of chemicals causing allergic contact dermatitis, addressed in this memorandum are the last of the CPSC-nominated activities regarding the LLNA.

This memorandum not only discusses the ICCVAM recommendations regarding the LLNA's ability to categorize the potency of chemicals causing allergic contact dermatitis, but it also discusses whether these revisions are acceptable in the regulatory context for the purpose of

classification for labeling under the Federal Hazardous Substances Act (FHSA) (15 U.S.C. 1261–1278).

I. Introduction

A. Background

The National Institutes of Health Revitalization Act of 1993 directed the National Institute of Environmental Health Sciences (NIEHS) to establish a method and criteria for the validation and regulatory acceptance of alternative testing methods (Public Law No. 103-43, Section 1301). To accomplish these goals, NIEHS created an ad hoc Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), which was made permanent by the ICCVAM Authorization Act of 2000 (Public Law 106-545). The Committee is composed of representatives from 15 federal regulatory and research agencies, including the U.S. Consumer Product Safety Commission ("CPSC" or "the Commission"). These agencies generate, use, or provide information from toxicity test methods for risk assessment purposes. The duties of ICCVAM are to review, optimize, and validate new, revised, or alternative test methods that encourage the reduction, refinement, or replacement of the use of animals in testing. In addition, ICCVAM is to provide test recommendations to federal agencies and other stakeholders to facilitate appropriate interagency and international harmonization of toxicological test protocols. In 1998, the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) was established to assist ICCVAM in performing the activities necessary for the validation and regulatory acceptance of alternative test methods. ICCVAM submits test recommendations for a test method to federal agencies that require or recommend acute or chronic toxicological testing. According to Public Law 106-545, these agencies should promote and encourage the development and use of alternatives to animal test methods for regulatory purposes and ensure that any new or revised acute or chronic toxicity test method is valid for its proposed use under the mandate of the ICCVAM Authorization Act of 2000.

On June 30, 2011, ICCVAM forwarded to the Commission for action, recommendations regarding the usefulness of the LLNA in determining the potency of sensitizing chemicals. The CPSC needs to determine whether the proposed recommendations regarding the LLNA would be acceptable under the Federal Hazardous Substances Act (FHSA). Under the mandate of the ICCVAM Authorization Act of 2000, federal agencies have 180 days to identify any relevant test methods for which the ICCVAM test recommendations may be added or substituted, review such test recommendations, and notify ICCVAM if they will adopt the ICCVAM test recommendations. The Commission needs to respond to ICCVAM by January 2, 2012.

B. Validation of Alternative Methods

Validation of alternative methods is required before regulatory acceptance and use by federal agencies. In general, for an alternative method to be considered valid, it must be reliable (*i.e.*, the toxicity predictions of test substances are repeatable within the same laboratory and reproducible across/among different laboratories) and relevant (*i.e.*, the alternative test method is useful for measuring the biological effect of interest, such as sensitization).

The reliability and relevance of an alternative test method can be assessed from the statistical analysis of data. The relevance of an alternative test method can be determined by comparing the performance of the alternative test to the test that it is designed to replace. Typically, performance is evaluated by calculating the accuracy,[1] false positive rate,[2] false negative rate,[3] sensitivity,[4] or specificity[5] of the alternative test method. The reliability of the alternative test method can be determined from the reproducibility of test method results within and among laboratories.

C. FHSA Requirements

Cautionary labeling of hazardous household substances is mandated by the FHSA. Under the FHSA, to be a hazardous substance, a product must present one or more of the hazards enumerated in the statute, and it must have the potential to cause substantial personal injury or substantial illness during, or as a result of, any customary or reasonably foreseeable handling or use. The hazards are described below.

FHSA Definition of "Strong Sensitizer": "Strong sensitizers" are one of the seven hazards defined under the FHSA. The definition of "*strong sensitizer,*" which appears in section 2(k) of the FHSA (15 U.S.C. §1262(k), and is restated in 16 CFR 1500.3(b)(9)) is:

> *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.*

Five substances have been identified in the FHSA as strong sensitizers[6]: (1) paraphenylenediamine and products containing it; (2) powdered orris root and products containing it; (3) epoxy resin systems containing in any concentration ethylenediamine, diethylenetriamine, and diglycidyl ethers of molecular weight less than 200; (4) formaldehyde and products containing 1 percent or more of formaldehyde; and (5) oil of bergamot and products containing 2 percent or more of oil of bergamot. These designated compounds were

[1] Accuracy – the proportion of correct outcomes.
[2] False positive rate – the proportion of all negative substances that are falsely identified as positive.
[3] False negative rate – the proportion of all positive substances that are falsely identified as negative.
[4] Sensitivity – the proportion of all positive substances that are classified as positive.
[5] Specificity – the proportion of all negative substances that are classified as negative.
[6] 16 CFR §1500.13.

transferred to the CPSC's jurisdiction from the U.S. Food and Drug Administration when the CPSC was created.

Since its inception in 1972, the CPSC has not designated any substances to be strong sensitizers. However, in 1986, the Commission issued a rule clarifying the FHSA's "strong sensitizer" definition, with supplemental definitions as recommended by a Technical Advisory Panel on Allergic Sensitization (TAPAS).[7] The following supplemental definitions were intended to clarify the interpretation of the statutory definition of a "strong sensitizer":

> - *Sensitizer: 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.*
>
> - *Strong: In determining that a substance is a "strong" sensitizer, the Commission shall consider the available data for a number of factors. These factors should include any or all of the following (if available):*
>   - *Quantitative or qualitative risk assessment*
>   - *Frequency of occurrence and range of severity of reactions in healthy or susceptible populations*
>   - *The result of experimental assays in animals or humans (considering dose-response factors), with human data taking precedence over animal data*
>   - *Other data on potency or bioavailability of sensitizers*
>   - *Data on reactions to a cross-reacting substance or to a chemical that metabolizes or degrades to form the same or a cross-reacting substance*
>   - *The threshold of human sensitivity*
>   - *Epidemiological studies*
>   - *Case histories*
>   - *Occupational studies*
>   - *Other appropriate in vivo and in vitro test studies*
>
> - *Severity of Reaction: The minimal severity of a reaction for the purpose of designating a material as a "strong sensitizer" is a clinically important reaction. For example, strong sensitizers may produce substantial illness, including any or all of the following:*
>   - *Physical discomfort*
>   - *Distress*
>   - *Hardship*
>   - *Functional or structural impairment*
>
> *These may, but not necessarily, require medical treatment or produce loss of functional activities.*
>
> - *Significant potential for causing hypersensitivity: "Significant potential for causing hypersensitivity" is a relative determination that must be made separately for each substance. It may be based on chemical or functional properties of the substance,*

[7] 16 CFR §1500.3(c)(5).

documented medical evidence of allergic reactions obtained from epidemiological surveys or individual case reports, controlled in vitro or in vivo experimental assays, or susceptibility profiles in normal or allergic subjects.

- Normal living tissue: The allergic hypersensitivity reaction occurs in normal living tissues, including the skin and other organ systems, such as the respiratory or gastrointestinal tract, either singularly or in combination, following sensitization by contact, ingestion or inhalation.

While the FHSA does not require manufacturers to perform any specific battery of toxicological tests to assess the potential risk of chronic hazards, the manufacturer is required to label a product appropriately, according to the FHSA requirements. The exception is that if the product is a toy or other article intended for use by children and is a hazardous substance, then the product is, by definition, a banned hazardous substance, unless specifically exempted.[8] When determining whether a consumer product, which is composed of a mixture of substances, is a hazardous substance, the mixture should be tested—and not the individual components of the mixture—because synergistic or antagonistic reactions may lead to erroneous determinations concerning the toxic, irritant, and corrosive properties of the substance (16 CFR § 1500.5).

Sensitizers in Art Materials: In 1988, Congress amended the FHSA, to include the Labeling of Hazardous Art Materials Act (LHAMA) requirements. LHAMA requires a reviewing procedure for developing precautionary labels for all art materials. This amendment to the FHSA concerns chronic health hazards known to be associated with a product or product component when present in a physical form, volume, or concentration that presents the potential to produce a chronic health hazard as determined by a toxicologist. Within the regulation under the Act, a "sensitizer" is defined as *a substance known to cause, through an allergic process, a chronic adverse health effect which becomes evident in a significant number of people on re-exposure to the same substance.*[9] To protect users from known sensitizers found within art materials, each label shall contain a list of sensitizers that are present in sufficient amounts to contribute significantly to a known skin or respiratory sensitization.[10]

D. Past and Current Sensitization Testing

Historically, data on the sensitization potential of chemicals came from studies using human volunteers. Two tests for predicting whether a person will become sensitized to a substance are the Human Maximization Test (HMT) and the Human Repeat Insult Patch Tests (HRIPT).[11] The HMT is no longer in use due to ethical concerns about its potential to create adverse health consequences for the person being tested. Contract laboratories have performed the vast majority of human sensitization tests. There are a limited number of scientific publications with human sensitization data, of which much is from older studies. The development of animal

[8] 16 C.F.R. §1500.3(b)(15)(i).
[9] 16 C.F.R. §1500.14(b)(8)(i)(B)(9).
[10] 16 C.F.R. §1500.14(b)(8)(i)(E)(5).
[11] These tests vary with regard to the number of induction patch tests, the placing of the patches, and the use of a maximization step (an amplifying step during the challenge phase, this step involves co-treatment of the test sensitizer of interest with an irritant in order to enhance a potential response).

sensitization tests has been based on a comparison of the human tests performed with the same chemicals.

Prior to development of the LLNA, the Guinea Pig Maximization Test (GPMT) and the Buehler Assay (BA) had been the primary animal assays used to determine the sensitizing ability of a chemical. The GPMT is a highly sensitive method; however, some of the sensitivity arises due to the coadministration of a painful immune stimulant. This method involves injecting under the skin of the animal the possible sensitizer being tested, as well as applying it to the surface of the skin. The BA uses repeat closed topical applications (filter papers containing the test sensitizer of interest are covered with a patch and taped to the skin in order to enhance absorption of the substance). The GPMT is regarded as a more sensitive assay that may also, for certain substances, overestimate the sensitization hazard for the compound tested. The BA is less sensitive and may underestimate the sensitization potential of a compound.

In 1997, the LLNA was proposed to ICCVAM as a standalone alternative method to the GPMT and the BA for sensitization hazard identification.[12] In 1999, based on the validation database and performance of the test method, ICCVAM recommended the LLNA as an alternative test method for assessing the skin sensitization potential of most types of substances. The consensus of the scientific peer review panel regarding the ICCVAM recommendation was that the LLNA performed as well as the GPMT and BA for hazard identification of strong to moderate chemical sensitizing [dermal] agents but lacked strength in accurately predicting some weak sensitizers and some strong irritants. The LLNA provides several advantages compared to the guinea pig assays, including elimination of potential pain and distress, use of fewer animals, shorter test duration, a more objective end point, less test substance required, and the availability of dose-response information. U.S. regulatory agencies (including the CPSC) accepted the LLNA as a valid alternative test method for allergic contact dermatitis testing. The LLNA also was adopted as a test guideline (test guideline [TG] 429) in 2002, by the Organization for Economic and Cooperative Development (OECD) after the ICCVAM validation of the assay.

In the intervening years, the National Toxicology Program (NTP) has used the assay extensively to study chemical hypersensitivity based upon its acceptance as a standalone alternative.

II. Alternative Tests for Sensitization, ICCVAM Recommendations

Currently, no *in vitro* or *in silico*[13] systems have undergone validation for determining sensitizing potential. Both approaches are evolving methodologies and are being pursued to reduce the number of expensive laboratory and animal experiments performed. There are four *in vitro* test methods that are in pre-validation review in the European Union. It is expected that none of these methods, each relating to a specific mechanistic step occurring in skin sensitization, will be accepted as a standalone method and instead will be part of an integrated testing strategy. The validation of some of these *in vitro* methods may be completed in 2012.

[12] The LLNA provides a yes/no answer about whether a substance is a sensitizer.

[13] *In silico* data is a computational approach, using sophisticated computer models for the determination of a sensitizing potential.

The remainder of Section II of this memo will describe the submitted ICCVAM recommendations, relevant validation and performance data, and ICCVAM conclusions. In March 2008, an international peer review panel (Panel) composed of expert scientists from industry, academia, and other scientific professionals,[14] convened to review and evaluate the validation status, make recommendations for revisions, and provide final comments on the usefulness and limitations of proposed modifications to the LLNA. The Panel provided conclusions and recommendations in its reports. ICCVAM subsequently considered the Panel's conclusions and recommendations, as well as comments from the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM)[15] and the public, updated the recommendations, and provided its recommendations to the federal agencies for their approval.

LLNA potency assessment

1. Background

The LLNA is a test method developed to assess the potential of a test substance to induce allergic contact dermatitis in humans. The basic principle underlying the LLNA is that sensitizers induce a proliferation of lymphocytes in the lymph node draining the site of substance application. Under appropriate test conditions, this proliferation is proportional to the dose applied and provides a means of obtaining an objective measurement of sensitization. The LLNA was the first test method evaluated and recommended by ICCVAM. As stated earlier, the advantages of this test method include that it uses fewer animals, provides dose-response information, and eliminates pain and distress compared to the guinea pig assays.

The GHS (Globally Harmonized System) is an internationally harmonized approach to classification and labeling for all chemicals and mixtures of chemicals. The CPSC is a member of the U.S. federal interagency work group participating in the development and possible implementation of the GHS. Respiratory and skin sensitizer classification is addressed in GHS chapter 3.4. Health Sciences (HS) staff was part of an OECD expert group formed to develop a revised GHS approach on sensitizers. The OECD sensitization expert group met for the final time in March 2008, at the CPSC, to continue work on the UN request for a proposal for revising the GHS chapter 3.4 with respect to strong versus weak skin sensitizers.[16] For the most part, agreement was reached, and the revised chapter was forwarded to the OECD Task Force on Harmonisation of Classification and Labelling. At its April 2008 meeting, this OECD Task Force agreed to the proposed revisions.[17] The revised sensitizer chapter was submitted as a formal

[14] This peer review panel was organized by ICCVAM, in collaboration with NICEATM.
[15] SACATM is a chartered advisory committee that 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.
[16] Thus, two expert groups (with some common participants), the OECD expert group and the ICCVAM Panel, were addressing issues on the LLNA, including the issue of sensitizer potency, at the same time.
[17] HS staff believes that the GHS approach for classifying and labeling chemicals that are sensitizers generally will be compatible with the FHSA "strong sensitizer" statutory and supplemental definition.

proposal to the UN Sub-Committee of Experts on the GHS and was accepted and adopted in December 2008.

Despite the agreement to move ahead with the revised GHS chapter, the issue of sensitizer potency,[18] and the tests that can be used to determine potency of chemicals that might be sensitizers, continued. European scientists favored the sole use of the LLNA for the determination of skin sensitizer potency. However, because of concerns about the scientific validity of the use of the LLNA to determine potency, CPSC nominated the LLNA test method, for determination of sensitization potency, to ICCVAM for its review. Specifically, ICCVAM was asked to review the validation status of the use of the LLNA as a standalone assay for the determination of potency. In March 2008, the ICCVAM Peer Review Panel (the Panel) recommended that the LLNA should be used as part of a weight-of-evidence approach for potency determinations, not as a standalone assay. With the ICCVAM analysis data and Panel recommendation in hand, CPSC staff persuaded its European counterparts on the OECD expert panel to agree that the revisions to the GHS sensitization chapter embrace the use of the LLNA as part of a weight-of-evidence approach, not as a standalone test. The final criteria adopted in 2008 by the GHS to distinguish strong skin sensitizers from other sensitizers, are based on appropriate human and/or experimental animal studies (*e.g.*, guinea pig and LLNA data). Substances with positive responses in the human tests (HMT or HRIPT) at induction thresholds ≤ 500 μg/cm^2, and/or with LLNA EC3[19] values ≤ 2%, and/or exceeding GPMT or BA thresholds (Appendix A) can be classified as strong skin sensitizers when subcategorization is desired by competent authorities.

2. Validation and Performance

NICEATM compiled a database of 466 substances tested in the LLNA (obtained from published sources and unpublished sources provided by regulatory agencies and manufacturers). A subset of the 466 substances had both human and LLNA data available so that the accuracy of the LLNA in identifying strong versus weaker sensitizers could be assessed (using the human data as the reference standard). For substances with more than one EC3 or human threshold value, the most conservative value was used in the 2007–2008 analysis.[20] NICEATM performed multiple comparisons between the LLNA EC3 data and human data with the human data sorted as:

- Human data of no observed effect levels (NOELs);

[18] Potency refers to the intrinsic property of a sensitizing chemical. Allergens are known to vary significantly (as much as 10,000-fold) in the potency in which they can induce skin sensitization. The more potent the substance, the smaller the quantity needed for sensitization induction (naïve exposure). Even though potency plays a large role in determining the threshold for induction or elicitation (previously exposed) of skin sensitization, other factors (*e.g.*, the extent of dermal exposure, the other mixture components) will affect these thresholds.

[19] EC3 is the estimated concentration of a substance expected to produce a stimulation index of 3.0, a threefold increase in lymphocyte proliferation, the threshold value used to discriminate between sensitizers and nonsensitizers in the LLNA.

[20] The slope of the regression was statistically different from zero ($p<0.001$), and the coefficient of determination (R^2) was 0.418, indicating that there is a positive correlation between LLNA EC3 and human threshold values.

- Human data of lowest observed effect levels (LOELs), divided by a safety factor of 10;
- Human data with respect to the (at the time) proposed "strong sensitizer" GHS thresholds of 250 $\mu g/cm^2$ and 500 $\mu g/cm^2$.

NICEATM determined that optimum EC3 values were 2.9 percent and 3.7 percent for the proposed GHS human threshold cutoffs of 250 $\mu g/cm^2$ and 500 $\mu g/cm^2$, respectively. The accuracy of the LLNA to predict strong human skin sensitizers ranged from 74 percent to 79 percent when false positives or false negatives were removed from the database; and decreased to 55 percent when false positives and false negatives were included in the analysis.[21]

3. Recommendations for the LLNA potency assessment

The Panel met in public session in March 2008, to review and evaluate the draft background review document and ICCVAM recommendations. The Panel provided final comments on the usefulness and limitations of the LLNA for potency determination in their May 2008 report. The Panel supported the draft ICCVAM recommendation that the LLNA should not be used as a standalone test method for categorizing substances as skin sensitizers based on potency, but that the test method instead be part of a weight-of-evidence evaluation. The Panel considered the database of substances analyzed to be representative of a sufficient range of chemicals and concluded that the accuracy analysis had made appropriate comparisons to the human data/experience; although one Panel member felt that the relevance of the LLNA to human clinical observations had not been sufficiently determined. The Panel also agreed that the two-level classification scheme (weak versus strong skin sensitizers) for both human and guinea pig data was appropriate, although a minority opinion by two Panel members was that a moderate category should be included because certain compounds might be on the border between weak and strong skin sensitizers. The Panel stated that:

- *The decision criteria providing the best overall performance was the use of <250 $\mu g/cm^2$ to distinguish between strong and weak sensitizers in humans and the use of a LLNA EC3 ≤ 9.4% to distinguish between strong and weak sensitizers in the LLNA. The Panel recommended a statistical analysis to determine where an appropriate cutoff value between weak or strong sensitizers might be best defined for LLNA data.*
- *Safety factors other than 10 for the lowest observed effect level (LOEL) should be evaluated to determine if improved results could be obtained. The Panel also suggested an analysis that directly compares the LOEL values with using a safety factor and an analysis that only uses no observed effect level (NOEL) data. The Panel recommended that the LOELs from Akkan (2003) be used instead of the DSA_{05} values from Schneider (2004) in all potency analyses. A minority opinion of one Panel member stated that it was acceptable to use the DSA_{05} values. This Panel member mentioned that the DSA_{05} value is a LOEL value adjusted to 5% incidence of induction in order to correct for human studies leading to different inductions.*

[21] Including the false positives and false negatives into the analysis also resulted in a shift in the optimum EC3 value to 5.9 percent.

- *The effect of vehicles should be recognized as a limitation in the data analyses and a likely source of intra- and inter-laboratory variability.*

Subsequent to the Panel meeting and public request for additional data, the NICEATM database increased to more than 600 substances tested in the LLNA. From this database, there were 136 substances for which both LLNA and human data (ranging in potency from substances determined to be nonsensitizers to strong sensitizers based on the human data) were available. To determine an optimum EC3 value that could be used to identify strong and other-than-strong sensitizers, NICEATM performed receiver-operator characteristic calculations. The optimum EC3 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 based on these criteria occurred at EC3 ≤ 3.8 percent and EC3 ≤ 3.5 percent.

As per the recommendation by the Panel to perform additional analyses using alternative human reference values, in the 2009–2010 analysis and final background review document, NICEATM converted the human data into terms of DSA_{05}.[22] This DSA_{05} value was deemed to be a human threshold response similar to the LLNA EC3 value. Geometric mean EC3 values were used for each substance as opposed to the earlier analyses using the most potent EC3.[23] Of the 136 substances, 76 substances were considered human skin sensitizers (from mild to stronger potency) based on the human test results. Using the human data as the gold reference standard, 52 percent (14/27) of the strong human skin sensitizers were also classified strong in the LLNA, while 41 percent (11/27) were classified as "other skin sensitizers." The majority (77%) of the under-classified substances produced a LLNA EC3 value between 2 percent and 10 percent. Compared to the human data, the LLNA under-classified 7 percent (2/27) of strong skin sensitizers and over-classified 6 percent (3/49) of "other skin sensitizers" as strong sensitizers.[24] For the 21 substances with an LLNA EC3 ≤ 2 percent, 67 percent (14/21) were classified as strong human skin sensitizers based on the human test data.[25]

4. ICCVAM Conclusion

ICCVAM concluded that the LLNA can be used to categorize substances as strong skin sensitizers when the estimated concentration that produces a positive LLNA response (EC3) is ≤ 2.0 percent.

[22] DSA_{05} is the dose per skin area that represents a 5 percent positive response among a study's test subjects.
[23] Using the geometric mean improved the coefficient of determination to 0.448 for the EC3 and DSA_{05} regression.
[24] The LLNA also over-classified 58 percent (35/60) of human nonsensitizers as skin sensitizers. For substances falling into the GHS subcategory 1B, other skin sensitizers, the LLNA correctly predicted the classification of 71 percent (35/49).
[25] Two of the five sensitizers identified in the FHSA as strong sensitizers (paraphenylenediamine and formaldehyde), are among the substances in the NICEATM database with both human and LLNA data. The LLNA correctly classified both substances as strong sensitizers based on the GHS criteria of an EC3 ≤ 2 percent.

III. ICCVAM Recommendations

ICCVAM concluded that the LLNA can be used to categorize substances as strong skin sensitizers. However, because some substances classified as strong sensitizers based on historical human data fell into the GHS subcategory 1B, "other sensitizers," ICCVAM recommended that the LLNA should not be considered a standalone assay for skin sensitization potency classification.

ICCVAM recommended the following future studies to characterize further 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 sensitizers based on a human threshold induction concentration of ≤500 $\mu g/cm^2$ to more accurately 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.*

IV. Discussion by CPSC Staff

Staff agrees with the ICCVAM recommendation that the LLNA should not be considered a standalone assay for skin sensitization potency classification.[26] Based on the strength of the analysis and the expanding database of LLNA data, this assay can be a valuable tool in a weight-of-evidence evaluation for skin sensitization potency of a substance.

Traditional regulatory test methods for skin sensitization focus on yes/no determinations of sensitization hazard. In 1999, the LLNA was validated as an alternative test method for assessing the skin sensitization potential of most substances. Beginning in the late 1990s, published studies analyzed whether the LLNA could predict and classify skin sensitizer potency. As mentioned previously, CPSC staff has been involved in both a U.S. federal interagency GHS work group, as well as an OECD expert group on sensitization, to develop a revised GHS approach on sensitizers with regard to skin sensitizer potency. The proposed criteria (and now finalized criteria) for the GHS skin sensitization sub-categorization consisted of the use of human, guinea pig, and LLNA data. During discussions among the OECD expert group, concern was voiced regarding the use of human data as the gold standard for the NICEATM/ICCVAM comparative analysis. Oral and written comments were submitted to ICCVAM regarding concern over this issue, that human data on skin sensitization thresholds has

[26] NICEATM also compared the performance of guinea pig tests for sensitization potency. The guinea pig tests classification rate was similar to the LLNA, although the LLNA correctly classified more strong skin sensitizers and other skin sensitizers.

been given undue status as an accurate gold standard. These views are represented in a written response by David Basketter, Frank Gerberick, and Ian Kimber, developers of the LLNA:

> *There is a paucity of data indicating the intrinsic potential of chemical skin sensitizers 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 judgment is inevitable to help compensate for the relatively poor quality of the limited human data that are available. It is our view that most of the variability in the dataset derives from the human studies.*[27]

NICEATM was unable to obtain the original records and/or compiled reports for all of the human reference data used in this evaluation. Ideally, all data supporting the validity of a test method should be obtained and reported from studies conducted in accordance with Good Laboratory Practice (GLP) guidelines, internationally recognized principles; however, a substantial portion of the human data is from studies performed in the 1960s, well before the establishment of GLP guidelines.

An issue that arose in discussions among the OECD expert group on sensitization, the ICCVAM Immunotoxicity workgroup (IWG), as well as the ICCVAM Panel was the potential impact of the LLNA vehicle on EC3 values, and therefore, potency classification. A vehicle can impact the skin's absorption of a test substance, and thus, potentially the immune response to that substance. NICEATM performed analysis on substances with data from tests in multiple vehicles. Based on the GHS subcategory classification system, potency classifications would differ for 18 percent (8/45) of these substances.

CSPC staff agrees with both the Panel's and ICCVAM's recommendation to continue to accrue data, because even though the NICEATM database is large for LLNA data (more than 600 substances), only 21 substances had EC3 $\leq$ 2 percent. What this does signify is that the majority of the available testing data consists of substances that are moderate, weak, or nonsensitizers, classes of substances that fall outside the CPSC's jurisdiction.

In 1984, the Commission adopted a policy to reduce the number of animals tested and to minimize the pain and suffering associated with testing (49 FR 22522). In addition, the use of laboratory animals is recommended in a tiered and sequential approach to testing. In a tiered-testing strategy, the test substance is tested *in vivo* if the appropriate hazard determination cannot be made from physicochemical characteristics, expert opinion, prior human experience, or prior animal testing. Under the FHSA, the determination of whether a substance is a "strong sensitizer" is based upon a weight-of-evidence approach. In the FHSA supplemental "strong sensitizer" definition, it is written:

> *Strong: In determining that a substance is a "strong" sensitizer, the Commission shall consider the available data for a number of factors. These factors should include any or all of the following (if available):*
>
> - *Quantitative or qualitative risk assessment*

[27] NICEATM performed analysis of covariance of variability for the geometric means of the human and LLNA data. There was similar variability for both sets of data (although a little broader for the human data).

- *Frequency of occurrence and range of severity of reactions in healthy or susceptible populations*
- *The result of experimental assays in animals or humans (considering dose-response factors), with human data taking precedence over animal data*
- *Other data on potency or bioavailability of sensitizers*
- *Data on reactions to a cross-reacting substance or to a chemical that metabolizes or degrades to form the same or a cross-reacting substance*
- *The threshold of human sensitivity*
- *Epidemiological studies*
- *Case histories*
- *Occupational studies*
- *Other appropriate in vivo and in vitro test studies*

Therefore, the LLNA would fit into a weight-of-evidence evaluation under the FHSA. Staff agrees with the ICCVAM Panel that the NICEATM analyses on the ability of the LLNA to determine sensitizing potency are based on sound science and are valid scientifically for their proposed uses.

V. Recommendation by CPSC Staff

Staff recommends accepting the ICCVAM recommendations. Staff agrees with the ICCVAM recommendation that the LLNA should not be considered a standalone assay for skin sensitization potency classification. Based on the strength of the analysis and the expanding database of LLNA data, this assay can be a valuable tool in a weight-of-evidence evaluation for skin sensitization potency of a substance.

Labeling of a consumer product regarding the hazards associated with that product is required by the FHSA. In order to determine the appropriate cautionary labeling for "strong sensitizers," animal testing may be necessary. However, the Commission supports minimizing the number of animals used and reducing the pain or suffering associated with animal testing and encourages the development and use of alternatives to animal test models. Thus, staff recommends that the Commission accept the ICCVAM recommendations because, although the ICCVAM 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 (based on the updated protocol), this application for potency determination could broaden the use of the LLNA protocol in place of guinea pig tests, and therefore, could reduce the number of guinea pigs that are being used to assess skin sensitization potency.

Following the Commission decision, staff will draft a letter to ICCVAM indicating the Commission's actions with regard to the ICCVAM recommendations. The ICCVAM website (http://iccvam.niehs.nih.gov/home.htm) will link to the Commission website, where we will post our acceptance or nonacceptance of the recommendations. In the section of the ICCVAM website, Pertinent Regulations, Guidelines and Laws (http://iccvam.niehs.nih.gov/agencies/regs.htm), there will be an announcement of the Commission's action on the acceptance or nonacceptance of the ICCVAM recommendations. Once ICCVAM receives responses from all the agencies, it will publish a *Federal Register* notice announcing all of the agencies' responses.

VI. Options

The Commission can vote to:

1. Accept the ICCVAM recommendations and instruct staff to draft a letter to ICCVAM indicating acceptance of its recommendations.

2. Reject the ICCVAM recommendations and instruct staff to draft a letter to ICCVAM indicating rejection of its recommendations.

References

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Appendix A

GHS Sensitization Cut-off Criteria

(a) The hazard categories for both respiratory and skin sensitizers consist of a Category 1 where subcategorization is not required by a competent authority[28] or where data are not sufficient for subcategorization. Where data are sufficient and where required by a competent authority, a refined evaluation allows the allocation into subcategory 1A, strong sensitizers, or subcategory 1B for other sensitizers (*e.g.,* moderate and mild sensitizers). The GHS building block approach allows a competent authority to select the appropriate elements for classification and communication according to their regulatory scheme (*i.e.,* selection of solely subcategory 1A or selection of both subcategories).

Hazard category and subcategories for respiratory/skin sensitizers

CATEGORY 1:	**Respiratory/Skin sensitizer**
	A substance is classified as a respiratory/skin sensitizer -if there is evidence in humans that the substance can lead to specific respiratory/skin hypersensitivity and/or -if there are positive results from an appropriate animal test.
Subcategory 1A:	Substances showing a high frequency of occurrence in humans and/or severity of reaction within an exposed population; or a probability of occurrence of a high sensitization rate in humans based upon animal or other tests.
Subcategory 1B:	Substances showing a low to moderate frequency of occurrence in humans and/or severity of reaction within an exposed population; or a probability of occurrence of a low to moderate sensitization rate in humans based upon animal or other tests.

In addition, when considering human evidence, the size of the population exposed and the extent of exposure are to be taken into account.

(b) Due to the large amount of data on the sensitizing strength of skin sensitizing chemicals, specific cutoff criteria are provided in the GHS chapter for skin subcategories 1A and 1B. Specific cutoff criteria are provided for human data and animal test results. The human evidence for a Subcategory 1A, **strong** sensitizer, can include positive results for the induction threshold at ≤ 500 $\mu g/cm^2$ in human diagnostic patch test data (specifically, the HMT and HRIPT). Animal test results for Subcategory 1A can include data from the LLNA and guinea pig tests (specifically, the GPMT and the BA). The cutoff values for the animal test results for Subcategory 1A, strong skin sensitizers, and Subcategory 1B, other skin sensitizers, are listed in the tables below.

[28] Examples of competent authorities would be CPSC, EPA, OSHA, and Health Canada.

Animal test results for Subcategory 1A, Strong Skin Sensitizers

Assay	**Criteria**
Local lymph node assay	EC3 value ≤ 2%
Guinea pig maximization 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

Animal test results for Subcategory 1B, Other Skin Sensitizers

Assay	**Criteria**
Local lymph node assay	EC3 value > 2%
Guinea pig maximization 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