July 1, 2004

MEMORANDUM

SUBJECT: Transmittal of Minutes of the FIFRA Scientific Advisory Panel Meeting Held May 4-6, 2004: A Consultation On Dermal Sensitization Issues For Exposures To Pesticides

TO: James J. Jones, Director
Office of Pesticide Programs

FROM: Paul I. Lewis, Designated Federal Official
FIFRA Scientific Advisory Panel
Office of Science Coordination and Policy

THRU: Larry C. Dorsey, Executive Secretary
FIFRA Scientific Advisory Panel
Office of Science Coordination and Policy

Joseph J. Merenda, Jr., Director
Office of Science Coordination and Policy

Please find attached the minutes of the FIFRA Scientific Advisory Panel open meeting held in Arlington, Virginia from May 4-6, 2004. These meeting minutes address a set of scientific issues being considered by the Environmental Protection Agency regarding a consultation on dermal sensitization issues for exposures to pesticides.

Attachment

cc:

Susan Hazen
Adam Sharp
Anne Lindsay
Janet Andersen
Debbie Edwards
Steven Bradbury
William Diamond
Arnold Layne
Tina Levine
Lois Rossi
Frank Sanders
Margaret Stasikowski
William Jordan
Douglas Parsons
Dayton Eckerson
David Deegan
Vanessa Vu (SAB)
Jack Housenger
Timothy McMahon
Jonathan Chen
Barbara Hostage
David Cooper
Elizabeth Hofmann
Michele Burgess
OPP Docket

Attachment

FIFRA Scientific Advisory Panel Members

Dr. Steven Heeringa
Dr. Stuart Handwerger
Dr. Gary Isom
Dr. Mary Anna Thrall
Dr. Paul Bailey
Dr. Gary Burleson
Dr. Ih Chu
Dr. Iain Foulds
Dr. A. Wallace Hayes
Dr. Abigail Jacobs
Dr. Jean Meade
Dr. Torkil Menne
Dr. Nancy Monteiro-Riviere
Dr. Richard Pleus
Dr. Paul David Siegel

SAP Report No. 2004-02

MEETING MINUTES

FIFRA Scientific Advisory Panel Meeting, May 4-6, 2004 held at the Holiday Inn Rosslyn Hotel at Key Bridge Hotel Arlington, Virginia

A Set of Scientific Issues Being Considered by the Environmental Protection Agency Regarding:

Consultation on Dermal Sensitization Issues for Exposures to Pesticides

NOTICE

These meeting minutes have been written as part of the activities of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), Scientific Advisory Panel (SAP). These minutes have not been reviewed for approval by the United States Environmental Protection Agency (Agency) and, hence, their contents do not necessarily represent the views and policies of the Agency, nor of other agencies in the Executive Branch of the Federal government, nor does mention of trade names or commercial products constitute a recommendation for use.

The FIFRA SAP was established under the provisions of FIFRA, as amended by the Food Quality Protection Act (FQPA) of 1996, to provide advice, information, and recommendations to the Agency Administrator on pesticides and pesticide-related issues regarding the impact of regulatory actions on health and the environment. The Panel serves as the primary scientific peer review mechanism of the EPA, Office of Pesticide Programs (OPP) and is structured to provide balanced expert assessment of pesticide and pesticide-related matters facing the Agency. Food Quality Protection Act Science Review Board members serve the FIFRA SAP on an ad-hoc basis to assist in reviews conducted by the FIFRA SAP. Further information about FIFRA SAP meeting minutes and activities can be obtained from its website at http://www.epa.gov/scipoly/sap/ or the OPP Docket at (703) 305-5805. Interested persons are invited to contact Paul Lewis, Designated Federal Official, via e-mail at lewis.paul@epa.gov.

SAP Report No. 2004-02

MEETING MINUTES:
FIFRA Scientific Advisory Panel Meeting, May 4-6, 2004, held at the Holiday Inn Rosslyn Hotel at Key Bridge Hotel Arlington, Virginia

A Set of Scientific Issues Being Considered by the Environmental Protection Agency Regarding:

Consultation on Dermal Sensitization Issues for Exposures to Pesticides

Mr. Paul Lewis
Designated Federal Official
FIFRA Scientific Advisory Panel
Date: July 1, 2004

Steven Heeringa, Ph.D.
FIFRA SAP Session Chair
FIFRA Scientific Advisory Panel
Date: July 1, 2004

Federal Insecticide, Fungicide, and Rodenticide Act
Scientific Advisory Panel Meeting
May 4-6, 2004

Consultation on Dermal Sensitization Issues for Exposures to Pesticides

PARTICIPANTS

FIFRA SAP Session Chair

Steven Heeringa, Ph.D., Research Scientist & Director for Statistical Design, Institute for Social Research, University of Michigan, Ann Arbor, MI

FIFRA Scientific Advisory Panel Members

Stuart Handwerger, M.D., Director, Division of Endocrinology, Cincinnati Children's Hospital Medical Center, University of Cincinnati, Cincinnati, OH

Gary E. Isom, Ph.D., Professor of Toxicology, Purdue University, School of Pharmacy & Pharmacal Sciences, West Lafayette, IN

Mary Anna Thrall, D.V.M., Professor, Microbiology, Immunology & Pathology, Colorado State University, College of Veterinary Medicine and Biomedical Sciences, Fort Collins, CO

FQPA Science Review Board Members

Paul Bailey, Ph.D., Senior Scientific Associate, Toxicology and Environmental Sciences Division, Intermediates and Synthetics Section, ExxonMobil Biomedical Sciences, Inc., Annandale, NJ

Gary Burleson, Ph.D., President, BRT-Burleson Research Technologies, Inc., Raleigh, NC

Ih Chu, Ph.D., Head, Systemic Toxicology and Pharmacokinetics, Environmental Science Bureau, Health Canada, Ottawa, ON, Canada

Iain Foulds, F.R.C.P., F.F.O.M. Senior Lecturer in Occupational and Environmental Dermatology,. The Birmingham Skin Centre, Sandwell and West Birmingham NHS Trust, United Kingdom

A. Wallace Hayes, PhD., DABT, FATS, FIBiol, FACFE, ERT, Visiting Scientist, Department of Environmental Health, Harvard School of Public Health, Harvard University, Boston, MA

Abigail Jacobs, Ph.D., Associate Director for Pharmacology and Toxicology, Offices of Drug Evaluation 4 and 5, CDER, FDA, Rockville, MD

Jean Meade, D.V.M., Ph.D., Toxicologist, Office of the Director, NIOSH, Morgantown, WV

Torkil Menne, M.D., Chair, Department of Dermatology, Gentofte Hospital, University of Copenhagen, Hellerup, Denmark

Nancy Monteiro-Riviere, Ph.D., Professor of Investigative Dermatology and Toxicology, Center for Chemical Toxicology Research and Pharmacokinetics, North Carolina State University, Raleigh, NC

Richard Pleus, Ph.D., Director and Toxicologist, Intertox, Seattle, WA; Adjunct Associate Professor of Pharmacology University of Nebraska Medical Center; Faculty member of the Center for Environmental Toxicology, University of Nebraska

Paul Siegel, Ph.D., M.S.P.H., Team Leader-Bioorganic Chemistry/Director Scientist, Analytical Services Branch, Health Effects Laboratory Division, NIOSH, Morgantown, WV

PUBLIC COMMENTERS

Oral statements were made by:

Michele Burgess, Ph.D., United States Environmental Protection Agency, Office of Solid Waste and Emergency Response

Mr. James Aidala, The Acta Group, L.L.C., representing Forest Products Research Laboratory

Howard Maibach, MD., University of California at San Francisco, representing Forest Products Research Laboratory

Susan Hunter Youngren, Ph.D., The Acta Group, L.L.C. representing Forest Products Research Laboratory

Mr. Dennis J. Morgan, representing Forest Products Research Laboratory

Paul A. Cooper, Ph.D., University of Toronto, representing Osmose, Inc.

Mr. John Horton, representing Osmose, Inc.

Ms. Deborah Proctor, Exponent, Inc., representing Tierra Solutions, Inc.

Joel Barnhart, Ph.D., representing Elementis Chromium

Mr. Warren Stickle, representing the Chemical Producers and Distributors Association

Jane Vergenes, Ph.D., International Specialty Products, representing the ACC Biocides Panel

Mr. Richard Wiles, representing the Environmental Working Group

Written statements were received from:

The ACTA Group, LLC

Beyond Pesticides and The Healthy Building Network

Joel Barnhart, Ph.D., Elementis Chromium, LLP

Michele Burgess, Ph.D., United States Environmental Protection Agency, Office of Solid Waste and Emergency Response

Forest Products Research Laboratory

Dr. Peter Griem, Clariant GmbH

INTRODUCTION

The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), Scientific Advisory Panel (SAP) has completed its review of the set of scientific issues being considered by the Agency pertaining to its review of dermal sensitization issues for exposures to pesticides. Advance notice of the meeting was published in the *Federal Register* on April 16, 2004. The review was conducted in an open Panel meeting held in Arlington, Virginia, from May 4-6, 2004. The meeting was chaired by Steven Heeringa, Ph.D. Mr. Paul Lewis served as the Designated Federal Official. Mr. Joseph J. Merenda, Jr. (Director, Office of Science Coordination and Policy,. EPA) and Mr. Jim Jones (Director, Office of Pesticide Programs, EPA) provided opening remarks at the meeting. Timothy McMahon, Ph.D. (Office of Pesticide Programs, EPA) discussed the proposed hazard identification methodology for assessment of dermal sensitization risk, and Jonathan Chen, Ph.D. (Office of Pesticide Programs, EPA) reviewed the proposed hazard identification methodology for assessment of dermal sensitization risk – a case study of Cr(VI) in wood preservatives. In preparing these meeting minutes, the Panel carefully considered all information provided and presented by the Agency presenters, as well as information presented by public commenters. This document addresses the information provided and presented within the structure of the charge by the Agency.

CHARGE

Dermal sensitization, also known as allergic contact dermatitis (ACD) is typically characterized by two phases, termed induction and elicitation. In the induction phase, the allergen is transported to regional draining lymph nodes where clonal expansion of allergen-specific T lymphocytes results. The elicitation phase results from a subsequent exposure to the allergen, in which the allergen-specific T-lymphocytes provoke a cutaneous immune response. Although several approaches have been proposed to assess threshold concentrations for induction and elicitation of ACD and risk determination for these concentrations, there is no established scientific approach within the Agency to do a quantitative risk assessment associated with ACD.

There are several accepted methods for hazard identification of dermal sensitization, including the Buehler occluded patch test, the guinea pig maximization test, and the murine local lymph node assay (LLNA). The guinea pig maximization test as well as the Buehler test, while providing reliable information on skin sensitization, are best suited for hazard identification. Several proposals have been published regarding quantitative determination of sensitization induction and elicitation thresholds.

ISSUE 1: Quantitative Risk Assessment for the Induction Phase of ACD

The Mouse Local Lymph Node Assay (LLNA) is a test method for assessing the allergic contact dermatitis (skin sensitization) potential of chemicals, specifically the induction phase of sensitization. Using the incorporation of radiolabeled thymidine or iododeoxyuridine into DNA, the LLNA measures lymphocyte proliferation in the draining lymph nodes of mice topically exposed to the test article. The stimulation index (ratio of lymphocyte proliferation in treated mice compared to controls) is used as the indicator of potential sensitization. In 1998, following review by the FIFRA SAP, the LLNA was incorporated as a screening test in OPPTS Test Guideline 870.2600 Skin Sensitization. In 1999, the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) Immunotoxicity Working Group (IWG) endorsed the LLNA as an acceptable alternative to currently accepted guinea pig test methods for hazard identification of chemicals with potential to produce contact hypersensitivity. Following additional studies to validate the method, the LLNA was endorsed by the SAP in December 2001 as a full stand-alone assay. The OPPTS guideline 870.2600 (Skin Sensitization) has been revised to include the LLNA as a stand-alone assay for appropriate applications. The OPPTS guideline has also been harmonized with OECD's Guideline 429 for LLNA, which was adopted in April 2002. Although the LLNA has not been validated for determination of sensitization potency, approaches for determination of quantitative assessment of sensitization induction thresholds have been proposed in the scientific literature (Gerberick 2000, 2001; Griem et al., 2003).

Gerberick (2000, 2001) proposed a methodology for determination of a 'sensitization reference dose' for sensitizers in consumer products. The lower boundary of the potency category for a sensitizing chemical is used as the starting point, with application of uncertainty factors for interindividual variability, product matrix effects, and use pattern. This approach was applied to the fragrance component cinnamic aldehyde and the preservative methylchloroisothiazolinone/methylisothiazolinone for which both LLNA and human

sensitization potency were available (Griem et al., 2003).

Griem et al (2003) proposed a quantitative approach in which identification of known human sensitizing chemicals used both an EC3 value (defined as the concentration of a sensitizer required to generate a threefold stimulation of proliferation in draining lymph nodes) from an LLNA test and a NOAEL or LOAEL from human repeat insult patch tests (HRIPT) or human maximization tests (HMT). The reported concentrations were converted into specific and molar area doses. Comparison of the area doses of the LLNA and human test results indicated that sensitization thresholds were similar in mice and humans despite the fact that the area doses for different chemicals ranged over several orders of magnitude (Griem et al., 2003). It was concluded from this analysis that the LLNA EC3 value is a useful measure of sensitizing potency in humans, and that the EC3 value can be used as a surrogate value for the human NOAEL that can be used as a starting point in quantitative risk assessment.

Uncertainty factors to account for interspecies variation, intraspecies variation, product matrix effects, and conditions of exposure (including repeated exposures) have been proposed for use in conduct of dermal risk assessments. Griem et al. (2003) have discussed the application and magnitude of all of these uncertainty factors with respect to establishment of safe area doses for both induction and elicitation, while Felter et al (2003) have proposed the use of only the intraspecies variation factor, product matrix factor, and exposure conditions factor for determination of safe area doses for induction. The interspecies uncertainty factor is intended to account for differences in response between tests in animals and results in humans, although it has been reported (Griem et al, 2003) that sensitizing area doses are very similar between murine and human data, thus supporting a potentially reduced uncertainty factor for this area. The intraspecies uncertainty factor is used to account for inherent variability in the human population based on age, sex, genetic makeup, or health status, and is generally agreed that a factor of 10 is appropriate for this uncertainty. An uncertainty factor may also be included for vehicle matrix effects, as the matrix in which an allergen is presented to the skin may have an influence on the potential for induction of ACD. Most experimental data are generated using simple vehicles, while actual exposures are usually to more complex formulations that may contain irritants or penetration enhancers. A factor of 10 may considered in such a case, while a reduced factor may be considered for mild formulations. Finally, an uncertainty factor may be applied to account for exposure variables that may influence the potential for induction of ACD, including the site of the body exposed, the integrity of the skin, and the potential for multiple exposures. Using the above approaches, a maximum uncertainty factor of 1000 or 3000 could be derived depending on the criteria used. By contrast, a minimum uncertainty factor of 10 could be derived if results from human studies are used.

Thresholds for induction of ACD can occur following a single exposure of sufficient magnitude, after contact with a large area of skin, or as a consequence of repeated skin applications (Marzulli and Maibach). Griem et al. (2003) suggested a possible higher sensitizing potency of a chemical upon repeated exposures. This would make sense in the case of hexavalent chromium, as the significant irritancy of the chemical could lend itself to an increased sensitizing potency by allowing more chemical to penetrate the stratum corneum.

QUESTION 1: What are the strengths and weaknesses of the proposed quantitative approach for

determination of induction thresholds to dermal sensitizing chemicals? What other approaches does the Panel recommend EPA consider? Which uncertainty factors does the Panel feel are the most appropriate for application to quantitative methods of induction threshold determination? What factors should be included in the determination of the magnitude of each uncertainty factor?

ISSUE 2: Quantitative Risk Assessment for the Elicitation Phase of ACD

Several proposals have been published regarding determination of elicitation thresholds in sensitized populations. The Minimum Elicitation Threshold (MET) concept has been discussed in previous publications (Nethercott et al., 1994; Zewdie, 1998; NJDEP, 1998; Basketter et al., 2003) specifically with respect to hexavalent chromium. The concept behind the MET is that there is an 'elicitation threshold' below which no sensitization reaction is expected; thus, the MET is analogous to an RfD (Horowitz and Finley, 1994). The setting of an MET is usually performed as a result of tests in previously sensitized individuals; thus, the MET is considered protective of elicitation reactions. However, there has not been an extensive discussion of the criteria for employing this concept for purposes of risk assessment.

QUESTION 2:

What are the strengths and weaknesses of the proposed quantitative approaches for determination of elicitation thresholds to dermal sensitizing chemicals? What other approaches does the Panel recommend that EPA consider? Which uncertainty factors does the Panel feel are the most appropriate for application to quantitative methods of elicitation threshold determination? What factors should be included in the determination of the magnitude of each uncertainty factor?

ISSUE 3: Children Sensitivity

Paustenbach et al. (1992) and Felter et al. (2002) have discussed the issue of whether children are more or less at risk for development of ACD. Paustenbach et al. addressed this issue specifically for hexavalent chromium, and this paper concluded that risk to children ages 3 to 8 is not likely to be greater than adults as there is no evidence that repeated exposures to hexavalent chromium places a person at greater risk of sensitization. Felter et al. suggested that infants and children may actually be at lower risk for development of ACD based on data gathered from dinitrochlorobenzene and pentadecylcatechol (poison ivy allergen). However, it is also understood that young children may not have been exposed to different allergens as compared to adults. In addition, increased frequency of exposure in children may increase the chance of induction to different allergens.

QUESTION 3:

Does the Panel agree that the available scientific data suggest no significant difference in the relative sensitivity of children vs. adults to the induction and/or elicitation of ACD? If so, please provide scientific justification for this position. If the Panel disagrees, please provide scientific justification, including supporting data and/or uncertainties in the explanation.

ISSUE 4: Case Example - Cr(VI) in treated wood

Data from murine LLNA tests as well as from human patch testing studies using hexavalent chromium are available in the scientific literature. Results of LLNA testing show EC3 values that indicate area doses that result in the induction of sensitization in the mouse, while the results of patch test studies in humans show area doses that result in elicitation of sensitization in already sensitized individuals. In the Agency's initial assessment seeking to assess dermal sensitization risk from hexavalent chromium, the lowest dose tested (0.018 ug/cm^2) from the human patch test study of Nethercott et al. (1994) was selected for determination of dermal risk from hexavalent chromium. A 10x uncertainty factor (3x for use of the lowest dose tested [LOAEL] in this study, and 3x to account for the small size of the study population in the Nethercott study) was applied, resulting in a 'safe area' dose of 0.0018 ug/cm^2. Use of the test data of Basketter et al. (2001) and Hansen et. al (2003) also result in derivation of similar 'safe' area doses of 0.001 and 0.003 ug/cm^2 respectively. Use of the murine LLNA test data and application of an uncertainty factor of either 1000 or 3000 calculated 'safe' area doses of 0.01 or 0.003 ug/cm2 respectively.

QUESTION 4: Please comment on the methods used for derivation of safe' area doses using the available LLNA data and the human patch test data, including the magnitude of the applied uncertainty factors, and include a scientific rationale in support of your position. Please comment on whether it is scientifically supportable to derive separate 'safe' area doses for protection against induction of dermal sensitization as well as elicitation in sensitized individuals by hexavalent chromium?

SUMMARY OF PANEL DISCUSSION AND RECOMMENDATIONS

- The Panel did not endorse any particular method for risk assessment related to the identification of thresholds for induction by dermal sensitizing chemicals but acknowledged the importance of incorporating all relevant data into the weight of evidence.
- Although the Panel sees promise in the use of the LLNA as a quantitative risk assessment tool, further development and validation of this application is necessary.
- The Panel proposed the following uncertainty factors be considered for the induction phase: interspecies variation (value of 1-10), intraspecies variation (value of 1-10), matrix/vehicle (value of less than 1 to 10), and exposure (value of less than 1 to 10).
- Given that sensitization responses are based on dose/surface area, the Panel concluded that both the Minimum Elicitation Threshold (MET) and LLNA exposure methodologies are appropriate for collecting sensitization data.
- The Panel identified four uncertainty factors (UF) for application to quantitative methods of elicitation threshold determination: interspecies variation, intraspecies variation, exposure, and vehicle/matrix.
- The Panel agreed with the Agency that there is no evidence of a significant difference in the sensitivity of children versus adults to the induction/or elicitation of ACD.
- Based on current exposure levels of hexavalent chromium in the environment, sensitivity in children is very rare.

- There are no data to suggest that allergic contact dermatitis occurs more frequently in children with atopic dermatitis compared to non-atopic children.
- Due to the availability of human data, there is no need at this stage to consider LLNA studies for the derivation of the 'safe' area dose (mass per unit area) for chromate exposure.
- The Panel identified the critical dose (lowest observed adverse effect level) [LOAEL] from the Nethercott et al. (1994) study should be 0.088ug/cm^2, which the Panel considered to still be a conservative safety level.
- Applying a matrix/vehicle (value 0.1), interspecies variation (value 1), intraspecies variation (value 1), and exposure (value 3 to 10) uncertainty factors, the S-RfD calculated as specific to hexavalent chromium in treated wood ranged from 0.09 to 0.3 ug/cm^2 .
- The Panel's estimate of an S-RfD should be protective against elicitation and therefore would also be protective of induction.
- Although the Panel calculated a S-RfD for hexavalent chromium in ACC treated wood, the Panel stressed that the Agency consider all data as part of a weight of evidence approach.

Panel Response To Question 1

General Comments

Before beginning to address the specific questions, the Panel offered some general comments relevant to the overall risk assessment process. Firstly, it was stated that there has been no established scientific approach within the Agency to conduct a quantitative risk assessment associated with allergic contact dermatitis (ACD) (Stern et al., 2003) and although several methods have been proposed and show promise, the Panel suggested that the weight of evidence approach remains appropriate at this time. The mechanisms of the induction and elicitation phases of ACD as well as the science underlying the proposed test methods will be discussed before evaluating the relevancy and validity of the proposed approaches. Each chemical has its own thresholds for induction and elicitation. For example, thresholds for Cr VI cannot be extrapolated to Cr III. Thresholds for induction of ACD can occur following a single dermal exposure of sufficient magnitude, after contact with the skin, or as a consequence of repeated skin applications (Marzulli and Maibach 1975). Griem et al. (2003) suggested a possible higher potential for inducing sensitization to a chemical upon repeated exposures.

ACD is characterized by two phases. The first is the induction phase which requires exposure of a susceptible individual to an allergen in sufficient concentration and for a sufficient duration to activate specific immune mechanisms that result in the acquisition of sensitization. During the induction phase, the allergen must penetrate the stratum corneum layers and be taken up by epidermal Langerhans cells which then process and transport the allergen to regional draining lymph nodes. Presentation of allergen results in clonal expansion of allergen-specific T lymphocytes and the generation of effector and memory T cells. The second phase is termed elicitation where re-exposure to the allergen (challenge) in a previously sensitized individual results in the elicitation of an inflammatory dermal response (Stern et al., 1993). As with induction, the elicitation phase requires penetration of the allergen through the stratum corneum and presentation by antigen presenting cells.

It is important when evaluating data derived from both animal and human studies to consider elements of the experimental design. Most animal assays used in the evaluation of ACD were originally designed for hazard identification. Guinea pig assays have been used for decades to predict the potential of chemicals to induce ACD in humans. The most frequently used tests are the Guinea Pig Maximization Test (GMT) and the Buehler test. These tests rely on the induction of sensitization in animals with the read out being the elicitation of a dermal inflammatory response following challenge. As the name would imply, the GMT provides for an exaggerated exposure regimen which includes intradermal injection of the allergen along with the use of an adjuvant and topical application under an occluded patch to maximize the potential to induce sensitization. In the Buhler test, animals undergo multiple exposures under an occluded patch. For the guinea pig assays, the end point is subjective and semi-quantitative.

More recently, a murine assay, the Local Lymph Node Assay (LLNA), has been developed which evaluates the induction/sensitization phase of ACD and provides for a dose response evaluation. Animals in this assay receive three consecutive days of topical exposure to intact, non-occluded skin. The read out is a quantitative measurement of ^{3}H-thymidine or I^{125} -deoxyuridine incorporation into draining lymph node cells as an indication of cellular proliferation. The LLNA has undergone intra- and inter-laboratory validation and peer-review sponsored by the ICCVAM (NIH 1999). The results of the ICCVAM review supported an equivalent percent accuracy for the LLNA and guinea pig assays in predicting the sensitizing potential of chemicals (~73%).

The Panel cited the strengths of the LLNA as:

- It is mechanistically-based, i.e. allergenic substances cause proliferation of lymphocytes;
- It provides an objective and quantitative endpoint;
- It permits the evaluation of ACD potential of chemicals that are too toxic to be tested in humans;
- It has been sufficiently validated for a hazard assessment;
- It reduces stress in animals due to the short duration of the assay, the open application of test material and the lack of the elicitation of the inflammatory response.

Deficiencies of the LLNA were described as:

- The irritant properties of the matrix or test substance may contribute to the local lymph node proliferation resulting in false positives for strong irritants;
- The method has not been sufficiently evaluated using mixtures;
- At the time of the ICCVAM review, its application had not yet been validated for metals or aqueous soluble materials (however since that time investigators have shown a similar predictive accuracy of the LLNA for metals (85%) as compared to low molecular weight chemicals (88%) (Basketter et al. 1999) and alternative vehicles for testing aqueous soluble materials have been investigated (Ryan et al., 2002).

Like the guinea pig assays, human tests to evaluate sensitization potential require the induction of sensitization and subsequent evaluation of the elicitation response following

challenge. Exposure under an occluded patch is used for induction in the Human Maximization Test (HMT) and the Human Repeated Insult Test (HRIPT), while the Open Epicutaneous Test utilizes topical application to non-occluded skin. The data from these tests are semi-qualitative and based on subjective scoring using macroscopic observation (i.e., +1 to +4). Although ethical issues may limit future human testing, the Panel felt strongly that when human data are available they should be given primary consideration.

Panel Conclusions/Recommendations in Response to Question 1.

Proposed quantitative approaches

The Panel did not endorse any particular method for risk assessment related to the identification of thresholds for induction by dermal sensitizing chemicals but acknowledged the importance of incorporating all relevant data into the weight of evidence. The Panel strongly agreed that given that the threshold for induction is considered to be higher than that required for elicitation (Friedman 1990), that establishing a safe level below the threshold for elicitation would also be protective of induction. Therefore it was anticipated that risk assessment for the induction phase of sensitization would only be appropriate for chemicals where no data were available (e.g. new chemicals). If risk assessment is based on induction only, exposure limits will be more conservative. If people are protected from induction, then elicitation should not be an issue (Gerberick and Robinson 2000). While there is basis for this hypothesis, there may be unanticipated exposure scenarios that could lead to a sensitized subpopulation.

In 1999, ICCVAM recommended that with certain protocol modifications, the LLNA was sufficiently validated as a stand-alone test for the identification of skin sensitizing chemicals with the exception of metals, mixtures, and the use of aqueous vehicles (National Institutes of Health 1999) . Since that time several authors have proposed the use of the LLNA for risk assessment. The first step was the analysis of dose response data to calculate an EC3 value (the concentration of chemical required to elicit a 3-fold increase in lymphocyte proliferation as compared to vehicle) as an estimation of the concentration of a chemical required to induce sensitization (Kimber and Basketter 1997). EC3 values of chemicals were then used to establish relative potencies. Potency data generated using this method compared favorably with the corresponding human data (Basketter et al. 2000; Gerberick et al. 2001).

More recent studies (Gerberick and Robinson 2000; Gerberick et al. 2001; and Griem et al. 2003) have incorporated LLNA derived EC3 values into quantitative risk assessment protocols. Gerberick et al. (2000, 2001) proposed a methodology for determining the 'sensitization reference dose' for sensitizers in consumer products. The lower boundary of the potency category for a sensitizing chemical was used as the starting point, with the application of uncertainty factors for interindividual variability, product matrix effects, and use pattern. This approach was applied to the fragrance component cinnamic aldehyde and the preservative methyl-chloroisothiazolinone/methylisothiazolinone for which both LLNA and human sensitization potency were available (Griem et al., 2003).

Griem et al. (2003) proposed a quantitative approach using both an EC3 value from the LLNA and a NOAEL or LOAEL from HRIPT or HMT. The reported concentrations were

converted into specific and molar area doses. The authors concluded from their analysis that the LLNA EC3 value was a useful measure of sensitizing potency in humans, and that the EC3 value can be used as a surrogate value for the human NOAEL and as a starting point in quantitative risk assessment.

Although the Panel sees promise in the use of the LLNA as a quantitative risk assessment tool, further development and validation of this application is necessary. Validation should include different classes of chemicals including metals. Academia, industry, and contract laboratories should participate in the validation process.

Uncertainty Factors

Uncertainty factors to account for interspecies variation, intraspecies variation, product matrix effects, and conditions of exposure (including repeated exposures) have been proposed for the conduct of dermal risk assessments. Griem et al. (2003) discussed the application and magnitude of all of these uncertainty factors with respect to establishment of safe area doses for both induction and elicitation, while Felter et al. (2003) discussed the use of only the intraspecies variation factor, product matrix factor, and exposure conditions factor for determination of safe area doses for induction. Uncertainty factors are generally assigned a value between 1 and 10. The interspecies uncertainty factor is intended to account for differences in response between tests in animals and results in humans. The intraspecies uncertainty factor is used to account for inherent variability in the human population based on age, sex, genetic makeup, or health status, and is generally agreed that a factor of 10 is appropriate for this uncertainty. An uncertainty factor may also be included for vehicle matrix effects, as the matrix in which an allergen is presented to the skin may have an influence on the potential for induction of ACD. Most experimental data are generated using simple vehicles, while actual exposures are usually to more complex formulations that may contain irritants or penetration enhancers. An uncertainty factor may be applied to account for exposure variables that may influence the potential for induction of ACD, including the site of the body exposed, the integrity of the skin, and the potential for multiple exposures. Using the above approaches, a maximum uncertainty factor of 1000 or 3000 could be derived depending on the criteria used. By contrast, a minimum uncertainty factor of less than 1 could be derived if proper human studies are conducted and used.

The Panel proposed the following uncertainty factors be considered for the induction phase: interspecies variation, intraspecies variation, matrix/vehicle, and exposure as described below. Uncertainty factors must be assigned on a case by case basis dependent in part on the experimental design from which the data were generated and the use of the chemical and product matrix.

Intraspecies variation - the Panel recommended a value for intraspecies variation of 1-10. The values depend on the experimental design of the study in question including factors such as the sample size, age, gender and ethnic composition of the sample population.

Interspecies variation - the Panel recommended values from 1 to 10. The values depend on the experimental design of the study in question. Rodent skin is much more permeable than

human skin for most compounds, potentially allowing enhanced penetration of the chemical and greater bioavailability (Tregear et al. 1975; Bartek et al. 1972). In addition, Griem et al. (2003) reported that sensitizing area doses are similar between murine and human data, thus suggesting a potentially reduced uncertainty factor for this area. Thus, this could result in an interspecies variation closer to 1.

Matrix/vehicle - this uncertainty factor is dependent upon the matrix used during testing as well as the anticipated matrix during use. Exposures during testing may have been exaggerated as compared to normal use. For example, exposure during patch testing may have been occluded for 48 hours. Additionally, DMSO, an irritant and skin penetration enhancer, is frequently used in the LLNA. Conversely, if mixtures were tested where the matrix included agents which may interfere with dermal penetration of the test article such as barrier creams or agents that may interfere with physiological responses such as vasoconstrictors or anti inflammatory agents, an uncertainty factor of greater than 1 would be expected. Therefore once the matrix used during testing and the anticipated matrix of exposure have been considered, the matrix/vehicle value assigned may range from less than 1 (e.g., DMSO) to 10 (e.g., dexamethasone).

Exposure - there is a need to consider the total dose when establishing an uncertainty factor for exposure. This may be dependent on the body site where exposure occurs as dermal penetration has been shown to vary between anatomical locations. The potential for repeat exposure and exposure to damaged skin must also be taken into consideration. Thus, the Panel recommended values from less than 1 to 10.

Panel Response To Question 2

Given that sensitization responses are based on dose/surface area, the Panel concluded that both the Minimum Elicitation Threshold (MET) and LLNA exposure methodologies are appropriate for collecting sensitization data (however, the LLNA does not measure elicitation). The use of the MET approach eliminates the need to extrapolate from animal data to humans. Variability with using this approach relates to the reliability of the patch test data (i.e. variability in defining threshold, the concentration of the patch test material, vehicle used, proper occlusion, consistency between readers, irritant responses, skin condition at patch site, patch time and the sample size). The issue related to sample size could be addressed by using 95% confidence limits. The 10% MET may not be an acceptable approach for new chemicals, however, since a sensitized population would not be available for testing, and ethical issues may prohibit the use of the HRIPT.

Although the LLNA method shows promise and data are being accumulated related to the use of the assay in risk assessment, the Panel does not feel that the method's suitability is yet adequately demonstrated. Validated methods for estimating elicitation thresholds do not currently exist. The Panel encourages the Agency to support research in methods development for use in risk assessment.

The Panel identified four uncertainty factors (UF) for application to quantitative methods of elicitation threshold determination: interspecies variation, intraspecies variation,

vehicle/matrix and exposure. For all uncertainty factors, determination of the magnitude should be assessed on a case by case basis. When animal models are used, it will be necessary to assign interspecies uncertainty factors. For mouse models, this UF should be on the low end of the 1-10 scale. Sensitization is based on dose per surface area and not on a mg/kg basis as with other toxicological effects and data have demonstrated a similarity in doses required to sensitize humans and mice (Gerberick et al. 2001). Additionally, dermal penetration is an important factor in the initiation of sensitization and, as stated previously,mouse skin is more permeable than human.

Intraspecies factors will be dependent on whether animal or human data are used for evaluation. When the extrapolation is from animal data to human, in addition to the interspecies factor, an intraspecies factor of 10 is generally used to account for differences in age, gender, ethnic background, genetic polymorphisms and skin condition. When human data are used, intraspecies uncertainty factors should take into account the study design and quality of the data, bearing in mind that the study population may consist of a sensitive subpopulation. The uncertainty factor should be dependent upon the number of people patch tested in relation to the percentage of individuals sensitized and the quality of the patch test data. The quality of the patch test data will depend on the expertise of the readers and consistency within and between centers. The determination of the threshold for interpreting a positive patch test reaction is variable. Additionally, an individual's irritant threshold, genetic factors, polymorphisms related to dermal metabolism, skin condition, and ultraviolet exposure may all influence their reaction.

Exposure factors must take into account repeat exposures, dermal integrity, potential for occlusion and anatomical site of exposure. The vehicle/matrix factors should take into account the matrix in which the chemical was tested as well as the vehicle/matrix anticipated during environmental exposure. This should include factors such as the irritant nature of the matrix, the presence of penetration enhancers or retardants and the bioavailability of chemical from the matrix.

Panel Response To Question 3

Background and Panel Recommendations On Clinical Aspects of ACD in Children

Compared to adults, ACD in children is rare. However this may be due to decreased exposure and not a difference in their immunologic response. The Panel agreed with the Agency that there is no evidence of a significant difference in the sensitivity of children versus adults to the induction/or elicitation of ACD. The Panel recommended the Agency review the article on this subject by Hjorth (1981). Historically, most cases of ACD in children have been caused by nickel, cobalt, fragrance, rubber additives and occasionally potassium dichromate. Most clinical cases in children are acute contact dermatitis settling rapidly with withdrawal from the allergen. Chronic dermatitis in children as a result of allergic contact factors is the exception. In recent years p-phenylenediamine has become a more frequent contact allergen in children as a result of temporary 'Henna' Tattoos. A single exposure to p-phenylenediamine from Henna tattoos can induce sensitization within 10 days, resulting in severe reactions at the site of exposure (Brancaccio et al 2002; Sidbury and Storrs 2000). The dermatitis experienced by children is equivalent to that experienced by adults. It involves any site exposed to an allergen and can

cause severe discomfort including itching, weeping, and pain. Significant morbidity can result.

Primary Sensitization In Children

Like adults, children can be sensitized with only one exposure to an allergen. P-phenylenediamine is one such example. Epstein et al (1961) demonstrated positive reactions to pentadecyl catechol (poison ivy) could be expressed in children of different age groups. This study suggested that not only can children be sensitized but that the rate of sensitization increases with increasing age. The rate of demonstrable sensitization rose from 30% in children under 1 year to 50% between 1 and 3 years. From ages 3 to 8 the rate rose further to 76%. However, the older children may have had increased environmental exposure to this common allergen with increasing age resulting in increased reaction rates. Thus, this study does not indicate that very young children are less likely to become sensitized.

Thimerosal reactions are common in young adults. Up to 15% of Swedish army recruits demonstrated this sensitivity (Hansonn and Moller 1971). This sensitivity is acquired from vaccinations preserved with thimerosal given in the early years of life.

Population Based Patch Test Studies

One cohort study has recently been performed by Mortz et al (2002). The study was designed to investigate both atopic disease and delayed type hypersensitivity. The cohort examined 1000 children aged 10 to 14 years old who were patch tested using the T.R.U.E test. 15.2% revealed patch test positive reactions. This figure was similar to that of an adult population (Neilsen and Menne 1992). The most common positive allergens were nickel, fragrances and thimerosal.

A study by Wohrl et al. (2003) reported 2776 consecutive patch tests given to a variety of age ranges. In the under 10-year-old age range, 62% gave positive reactions. In the over 70-year-old age range, a lower figure of 34.9% gave positive reactions. The study suggested that children may be more easily sensitized. However, this study makes no allowance for the selection of patients for patch testing in the first instance. There is often reluctance to patch test small children because of the discomfort of the testing, the small skin area available for testing and the cause of the ACD which is usually detectable from patient history. The patch test tends to be used in children to support a suspicion of sensitivity when confirmation is required and therefore when a child is tested it is more likely to be positive. In adults with persistent dermatitis, patch testing is more likely to be conducted in the hope of identifying a relevant allergen. Hence the incidence of positive patch test reactions in adults can be expected to be considerably lower.

Children's Exposure To Hexavalent Chromium And ACD

Based on current exposure levels of hexavalent chromium in the environment, sensitivity in children is very rare. Children around the world have for years been exposed to chromate in treated woods (CCA and ACC) and there are no reports in the literature of either sensitization occurring from this exposure or the elicitation of dermatitis in a sensitized child. Panel members

had collectively more than 50 years of patch testing experience, yet could not recall a single case of sensitization to hexavalent chromium being attributable to treated wood exposure either in children or in adults. Nor could they recall a single case of elicitation of ACD in hexavalent chromium sensitized children. One case was reported of an adult who had been previously sensitized to hexavalent chromium in the construction industry. This patient subsequently returned to work at a wood treatment plant, directly handled freshly treated wet wood and subsequently developed an elicitation reaction from this exposure. This resulted in removal of the patient from the source.

There are no data to suggest that allergic contact dermatitis occurs more frequently in children with atopic dermatitis compared to non-atopic children. Some studies actually suggest that the incidence of delayed type hypersensitivity is reduced in atopic children (Agner and Menne 2001). Therefore, children with active atopic skin disease should not be at any increased risk of ACD from hexavalent chromium in treated woods. This conclusion also applies when exposure might be expected to occur on actively inflamed skin.

Panel Response To Question 4

Introduction

ACD to hexavalent chromium may sometimes be very severe, with a major impact on the quality of life for some sensitized individuals. ACD can be a reversible condition if exposure is removed. In chromate dermatitis, the condition can persist when exposure is apparently removed (Wall 1980; Freeman 2000). As stated previously, there is no published literature to suggest that there is any primary sensitization or elicitation of ACD as a result of chromate exposure from treated woods.

LLNA Data

There were five studies cited by the Agency on the use of the LLNA and induction of chromate sensitivity (Griem 2003). However, as noted previously by the Panel, the LLNA has not been formally evaluated for use in risk assessment and issues still remain regarding the use of the LLNA in assessing metals. There are a number of well designed human studies in chromate sensitized individuals. Thus, due to the availability of human data, there is no need at this stage to consider LLNA studies for the derivation of the 'safe' area dose (mass per unit area) for chromate exposure.

Exposure Scenario for Chrome VI Case Study

A major concern in conducting a risk assessment on hexavalent chromium in treated wood is the paucity of exposure data. The Panel suggested that if exposure scenarios are to be considered, the following situations be considered:

- Wood treated with ACC (aged versus newly treated wood).
- Contact of treated wood used in the construction of structural components of outdoor play sets.
- Wood used in home patios, porches, decks, docks, etc.

- Woods treated with deck cleaners and bleaches.

Chromate Human Threshold Studies

The critical human chromate study conducted by Nethercott et al. (1994) was an elicitation study of an identified chromate sensitized population. This therefore represents a specialized subpopulation of the general population. Although there are no methods available to predict which individuals have a particular propensity for developing ACD to chromate, Nethercott et al. (1994) identified their subpopulation from a group of 6000 patients who had been patch tested. Of this group, 102 were re-patch tested to hexavalent chromate and 54 were confirmed to be chromate sensitive. This is therefore a significantly large population of sensitized individuals. The study had a good experimental design with several rounds of testing to not only reconfirm sensitivity but also to establish a dose-response relationship for elicitation. The hexavalent chromium sensitized subpopulation was identified using the T.R.U.E. test, a sensitive patch test system for metals. The Nethercott et al. (1994) study was conducted under occlusion and can be considered to be conservative.

The Panel also reviewed other chromium studies. The Hansen et al. (2002; 2003) study was supportive but not large enough to be considered by itself as there was a smaller subpopulation of 17 hexavalent chromium sensitized individuals compared to the Nethercott et al. (1994) study. The study by Fowler et al. (1999) did not have a good experimental design and therefore cannot be considered as a critical study. Other studies cited were reviewed (e.g. Hansen et al. 2002) and these are also supportive but have larger thresholds for elicitation of reactions. If designed for risk assessment purposes, a more appropriate study should have been undertaken over a period of at least four weeks, performed as an Open Test with repeated daily exposures.

The Critical Dose (LOAEL)

The Panel identified the critical dose (lowest observed adverse effect level) [LOAEL] from the Nethercott et al. (1994) study should be 0.088ug/cm^2, which the Panel considered to still be a conservative safety level. This dose was in contrast to the 0.018ug/cm^2 suggested by the Agency. At 0.088ug/cm^2 reactions occurred in 4 out of 54 subjects tested, representing about 10% of the hexavalent chromium sensitized subpopulation and equivalent to a MET 10.

Uncertainty Factors Considerations

Areas of uncertainty are considered when extrapolating the result of the critical dose to conditions relevant to the human exposure of interest. In this case, the Panel assessed human exposure to wood treated with hexavalent chromium. As presented previously by the Panel, the areas of uncertainty that have been identified for dermal risk assessment are: (1) interspecies variation; (2) intraspecies variation; (3) vehicle or product matrix effects; and (4) exposure considerations (i.e., area of the body exposed, repeated exposures). For each of these four areas, a range of values less than 1 to as high as 10 were chosen. A summary of the values chosen for each UF is presented in Table 1.

Given that the Nethercott et al. study (1994) was conducted in humans and there was no need for an UF for interspecies variation, a value of 1 was assigned. As the subjects in Nethercott et al. (1994) were sensitized to hexavalent chromium, these individuals represent a subpopulation of the normal population, which would have a reaction to hexavalent chromium. This subpopulation is more sensitive to induction and subsequent elicitation to hexavalent chromium. Thus for intraspecies UF, a value of 1 was chosen as a conservative estimate.

Another major area for data extrapolation involves the matrix in which the chemical is present and how the individual was exposed (Felter et al. 2002). The product matrix may affect the permeability of the skin such that there may be an enhancement or inhibition of the chemical penetration into the skin. This includes such things as irritants, penetration enhancers or inhibitors etc. The subjects in the Nethercott et al. (1994) study were tested to hexavalent chromium in occluded patches for a continuous 48 hour period. Patch testing with occlusion is designed to maximize penetration of the test substance. Thus for the matrix UF, a value of 0.1 was chosen to account for the more artificial situation in the Nethercott et al. (1994) study relative to actual exposures to wood treated with hexavalent chromium. A value of less than 1 was considered appropriate where the test matrix is likely to induce enhanced penetration and or augment the induction/elicitation process relative to the matrix of environmental concern. As the Panel has presented in response to this and previous questions, uncertainty factors range from 1 to 10 (Dourson et al. 1996). The Panel is cognizant their selection of an uncertainty factor less than 1 deviates from traditional uncertainty factor analyses. However, based on the conditions presented by the Panel, the Panel believed that an uncertainty factor less than 1 was appropriate for the matrix effect.

The Nethercott et al. (1994) exposure assessment provides for an estimate of the dermal exposure to the test substance in units of ug/cm^2. Such factors as site of body exposed, effect of occlusion, dermal integrity and certain environmental conditions were included in the Panel's determination of an exposure UF. The real world exposure to hexavalent chromium would consist of short-term repeated dermal exposures. Typically, most potential dermal exposures would be prevented by barriers people would have as a part of their normal life, such as clothing, shoes, and towels. However, it is likely that hands and feet can come into short term and repeated contact to treated wood. On decks with built in furniture, people would have dermal exposure on the legs (e.g. back of the thighs and calves) if they are wearing short pants. Furthermore the exposure to hexavalent chromium could be enhanced from routine cleaning of decking with alkaline-based deck-cleaning products. The Panel's determination of an exposure UF accounts for the differences between conditions in the Nethercott et al. (1994) study and those conditions likely to be encountered for ACC hexavalent chromium treated wood products. Thus, for the exposure UF a value ranging from 3 to 10 was chosen to account for real world repeated exposures that would occur to wood treated with hexavalent chromium.

Table 1. Summary of Uncertainty Factors

Condition	Uncertainty Factor
Matrix/vehicle	0.1
Interspecies variation	1
Intraspecies variation	1
Exposure	3 to 10

Estimated S-RfD

S-RfD is a conservative estimate, with associated uncertainty, of a dermal exposure (in units of ug/cm^2) that would not be expected to result in the induction of sensitization in the general population, including more responsive subpopulations (Felter et al 2003). The minimum elicitation threshold (MET 10%) is the concentration that would elicit an allergenic reaction in 10% of a sensitized population. As described previously, the Panel selected a MET 10 of 0.088 ug/cm^2 from the Nethercott et al. (1994) study for use in calculating a S-RfD. Applying the uncertainty factors presented in Table 1 (uncertainty factors ranged from 0.3 to 1), the S-RfD calculated as specific to hexavalent chromium in treated wood ranged from 0.09 to 0.3 ug/cm^2 . This calculation is presented in Figure 1.

Figure 1. Calculation of S-RfD

$$\frac{0.088 \text{ ug/cm}^2}{(.1)\ (1)\ (3 \text{ to } 10)} = \frac{}{[.3 \text{ to } 1]} \quad 0.3 \text{ to } 0.09 \text{ ug/cm}^2$$

The Panel's estimate of an S-RfD should be protective against elicitation and therefore would also be protective of induction, as thresholds for induction are generally higher than those for elicitation (Kimber et al., 2003). Although the Panel calculated a S-RfD for hexavalent chromium in ACC treated wood, the Panel stressed that the Agency consider all data as part of a weight of evidence approach.

Panel Consideration Of Relationship Of Environmental Media And The Acceptable Area Dermal Dose

During the public comments section of this FIFRA SAP meeting, the EPA, Office of Solid Waste and Emergency Response, presented comments on the relationship of environmental media (e.g. soil, wood or water) and acceptable area dermal dose. While the Panel was not explicitly charged with responding to the issues presented by EPA OSWER, they decided that such issues require consideration by the FIFRA SAP. EPA OSWER's questions to the Panel and the Panel's response are presented below.

EPA OSWER Questions

(1) Does the Panel agree that environmental matrix variables will influence the acceptable area dermal dose to induce/elicit contact dermal sensitization in an individual when exposed to a

chemical incorporated in an environmental media?

(2) Please describe how media-specific characteristics have or do not have a substantial impact on determining an environmental acceptable dermal dose for a chemical incorporated in soil, wood, and water matrices.

Panel Response

The FIFRA SAP agreed that matrix (wood, soil, water) variables can influence the amount of compound available for penetration through the stratum corneum bilipid layers to have an effect. Thus, the use of uncertainty factors are applied to account for this variation. For example, wet cement is different from cement powder. Aqueous solutions can hydrate the stratum corneum, thereby increasing the rate of penetration of a compound through the skin. The pH of the matrix can also influence the penetration of a compound and change the charge of a compound. Fixation of a compound in an environmental medium can lower the bioavailability of that compound.

Media-specific characteristics can play an important role in assessing the bioavailability of a compound. The physical/chemical characteristics, including potential for the matrix to cause dermal irritation, may contribute to or decrease a compound's ability to cause dermal induction/elicitation. Metal speciation of the compound in the matrix is also important, for example Cr (VI) is more toxic than Cr (III) and therefore poses a greater hazard. It is not important how much of a chemical is in the matrix but how much is leached out of it and available for exposure. All of these factors can influence how much material/compound would be available to have an effect.

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