Appendix G3

Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) Comments SACATM Meeting on June 18–19, 2008

The following is excerpted from the final minutes and speaker presentations of the SACATM meeting convened on July 18-19, 2008. The full meeting minutes are available online at http://ntp-server.niehs.nih.gov/?objectid=AF6CC417-F1F6-975E-75B5F3FF7DF1CDDC.

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Validation Status of New Versions and Applications of the Murine Local Lymph Node Assay

Dr. Marilyn Wind presented the "Report on the Independent Scientific Peer Review Meeting: Validation Status of New Versions and Applications of the Murine Local Lymph Node Assay (LLNA), a Test Method for Assessing the Contact Dermatitis Potential of Chemicals and Products – Introduction and Overview," authored by Dr. Joanna Matheson.

In 2007, the timeline for the ICCVAM evaluations included the nomination from the CPSC, endorsement by ICCVAM, and SACTAM's endorsement of the recommendation. In 2008, the LLNA Peer Review Panel met and a report was made available. The new/updated LLNA applications and protocols reviewed by the Peer Review Panel included:

- LLNA limit dose procedure
- LLNA for testing mixtures, metals, and aqueous solutions
- Non-radioactive LLNA: DA method
- Non-radioactive LLNA: BrdU-FC method
- Non-radioactive LLNA: BrdU-ELISA method
- Draft ICCVAM LLNA performance standards
- LLNA for potency determinations.

The documents prepared by the NICEATM and ICCVAM Immunotoxicity Working Group (IWG) for each new/updated LLNA applications included the draft background review document (BRD), the draft ICCVAM test method recommendations (usefulness and limitations, recommended protocol, future studies), and questions for the Peer Review Panel.

Dr. Wind presented an overview of the LLNA test method protocol.

- The LLNA protocol was initially described by Kimber et al. (1986).
- The purpose of the LLNA is to identify chemical sensitizers through quantification of lymphocyte proliferation.
- The LLNA uses a minimum of three dose levels. The highest dose level should be the maximum soluble concentration that does not cause systemic toxicity or excessive local irritation.
  - A stimulation index (SI) is calculated as the ratio of radioactivity incorporated into the cells of auricular lymph nodes of the treated animals to that of the vehicle control animals. The threshold for classifying a substance as a skin sensitizer is an $SI \geq 3$.
  - In order for an LLNA study to be considered acceptable, the concurrent positive control must yield an $SI \geq 3$.

A condensed version of the LLNA test method protocol was provided.

- Test substance is applied to mouse ears on Days 1, 2, and 3.
- On Day 6, mice are injected with radiolabeled thymidine (or an analogue of thymidine).
- Radiolabeled thymidine is incorporated into the DNA of proliferating cells and the auricular lymph nodes are removed.
- The amount of radiolabeled thymidine in the lymph nodes is determined as a measure of lymphocyte proliferation. The ratio of incorporated radioactivity in the auricular lymph nodes of treated vs. control mice (i.e., SI) is calculated, which leads to classification of a compound as negative or as a sensitizer.

The sole difference between the LLNA limit dose (rLLNA) test method protocol and that of the traditional LLNA protocol is that only a single dose, the highest dose that does not induce systemic toxicity or excessive local irritation, is used.

The LLNA Limit Dose Test Method Database contains information that is included in the BRD, which is based on a retrospective review of traditional LLNA data that were either submitted as part of the original LLNA evaluation (ICCVAM 1999), extracted from peer-reviewed publications, or submitted to NICEATM in response to an *FR* notice requesting available data and information. Data from 471 studies representing 466 unique substances are available (211 substances were included in the 1998 ICCVAM evaluation of the traditional LLNA). Results with the LLNA limit dose test procedure almost always agree with results from the traditional LLNA. Kimber et al. (2006) showed a 98.6% accuracy for 211 substances, and ICCVAM (2008) showed a 98.9% accuracy for 466 substances.

Dr. Wind provided the draft ICCVAM recommendations for the LLNA Limit Dose Test Method.

- The LLNA limit dose procedure should be used for the hazard identification of skin sensitizing substances if dose response information is not needed. Use all other LLNA protocol specifications recommended by ICCVAM (ICCVAM 1999, Dean et al. 2001).
- Users should be aware that the limit dose is the highest soluble concentration that does not induce overt systemic toxicity and/or excessive local irritation. A small possibility of a false negative result exists (1.6% [5/313]) when compared to the traditional LLNA.

Overview of the Peer Review Panel Report

Dr. Michael Luster presented the "Overview of the LLNA Independent Scientific Peer Review Panel Report," starting with the charge to the Panel to review the draft BRDs and to evaluate the extent to which applicable validation and acceptance criteria of toxicological test methods have been appropriately addressed. Further the Panel was to consider the ICCVAM draft test method recommendations for proposed method uses and limitations, recommended

standardized protocols, test method performance standards, and proposed future studies. The Panel was to then comment on the extent to which these items are supported by the information provided in the BRD. The Panel evaluated the LLNA modifications and applications as listed above.

Dr. Luster provided highlights of the final Independent Scientific Peer Review Panel report and recommended that the report should be consulted for a detailed description of the Panel's conclusions and recommendations. He provided evaluation highlights for all LLNA modifications and applications. The following points were made about the LLNA Limit Dose Procedure (rLLNA).

- The procedure follows the traditional ICCVAM LLNA protocol except for the number of doses tested. This procedure uses only the high dose group (requires 40% fewer animals).
- In general, the Panel concurred with the draft ICCVAM recommendation that the LLNA limit dose procedure should be routinely recommended for the hazard identification of skin sensitizing chemicals when dose response information is not required. The Panel also recommended that the limit dose procedure can be used as an initial test when dose-response information is required.
- The Panel also recommended that if dose-response information is required, as a way to further reduce animal use, the LLNA limit dose procedure should be routinely recommended as the initial test to identify sensitizers before conducting the traditional LLNA since negative results would not require further testing.
- Additionally, the Panel suggested that the test be referred to as the "reduced LLNA" (rLLNA) to be consistent with ECVAM terminology.

SACATM Discussion

There were no public comments specific to the rLLNA.

Dr. Grantley Charles concurred with the recommendation that the rLLNA protocol should include a discussion of how to determine the maximum dose if only a single dose is to be used in a screen process. An investigator must be able to define excessive irritation; otherwise, the testing may produce a bell-shaped response curve. The updated ICCVAM LLNA protocol has been revised to add specific guidance on determining the maximum concentration to be tested to avoid overt systemic toxicity and excessive local irritation.

Dr. Marion Ehrich suggested that the rLLNA appears favorable because 153/153 nonsensitizing agents and 308/318 sensitizing agents were predicted.

Dr. Luster said that the Panel made a very strong suggestion at the panel meeting that there be some histology associated with the rLLNA procedure. Histology was part of the plan, and it is embedded in the text.

Dr. Donald Fox stated that use of the reduced LLNA procedure could encourage the alternative use of animals.

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