Preface

The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) is charged by the ICCVAM Authorization Act of 2000 with evaluating the scientific validity of new, revised, and alternative toxicological test methods applicable to U.S. Federal agency safety testing requirements (ICCVAM 2000). ICCVAM is required to provide recommendations to U.S. Federal agencies regarding the usefulness and limitations of test methods based on this scientific evaluation. This Test Method Evaluation Report provides ICCVAM recommendations for five *in vitro* test methods proposed for assessing the potential pyrogenicity of pharmaceuticals and other products. These recommendations are based on a comprehensive evaluation of the current validation status of these test methods.

In March 2005, the European Centre for the Validation of Alternative Methods (ECVAM), a unit of the Institute for Health and Consumer Protection at the European Commission's Joint Research Centre, submitted background review documents (BRDs) to ICCVAM for five *in vitro* test methods, which were proposed as replacements for the rabbit pyrogen test. The information in the BRDs was based on validation studies financed by the European Commission within the 5th Framework Programme of Directorate General Research, the results of which were recently published (Hoffmann et al. 2005a; Schindler et al. 2006). The five test methods are:

- The Human Whole Blood (WB)/Interleukin (IL)-1β *In Vitro* Pyrogen Test
- The Human WB/IL-1β *In Vitro* Pyrogen Test: Application of Cryopreserved Human WB
- The Human WB/IL-6 *In Vitro* Pyrogen Test
- The Human Peripheral Blood Mononuclear Cell/IL-6 *In Vitro* Pyrogen Test
- The Monocytoid Cell Line Mono Mac 6/IL-6 *In Vitro* Pyrogen Test

In June 2005, ICCVAM initiated evaluation of the validation status of these five test methods. An ICCVAM Pyrogenicity Working Group (PWG) was established to work with the National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) to carry out this review. Dr. Marlies Halder was designated by ECVAM as their liaison to the PWG. Following a NICEATM pre-screen evaluation of the ECVAM BRDs, NICEATM, ICCVAM and the ICCVAM PWG requested additional information and clarification from ECVAM on a number of issues. In March 2006, ECVAM provided revised BRDs and responses addressing these issues.

NICEATM, in conjunction with the PWG, prepared a comprehensive BRD to combine the available data and information for each of the five *in vitro* test methods into one document. The ICCVAM BRD describes the current validation status of these test methods, including what is known about their reliability and accuracy, the scope of the substances tested, and the availability of standardized protocols for each test method. The ICCVAM BRD was based on the ECVAM BRDs, but also includes other relevant data and analyses, including data and information submitted to NICEATM in response to a *Federal Register* (*FR*) Notice (Vol. 70, No. 241, pp. 74833-74834, December 16, 2005). The ICCVAM draft BRD was made available to the public on December 12, 2006 (announced in *FR* Vol. 71, No. 238, pp. 74533-

74534, December 12, 2006) for comment and a public peer review panel meeting on February 6, 2007 was announced.

The independent scientific peer review panel (Panel) met in public session on February 6, 2007 at the National Institutes of Health in Bethesda, Maryland. The Panel first reviewed the ICCVAM draft BRD for errors and omissions and then discussed the current validation status of the five *in vitro* test methods. The Panel also reviewed the extent that the information in the ICCVAM BRD supported the ICCVAM draft test method recommendations for proposed test method uses, standardized protocols, test method performance standards, and future studies. Throughout the review process, interested stakeholders from the public were provided opportunities to provide comments including oral comments at the Panel meeting. The Panel considered these comments as well as public comments submitted in advance of the meeting before concluding their deliberations. The final independent Panel report was made available to the public (http://iccvam.niehs.nih.gov/docs/pyrogen/PrRevPanFinRpt.pdf) for review and comment on May 9, 2007 (announced in *FR* Vol. 72, No. 89, pp. 26395-26396).

The ICCVAM draft BRD and draft recommendations, the Panel report, and all public comments were made available to ICCVAM's advisory committee, the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM), and comments were provided at their meeting on June 12, 2007.

ICCVAM and the PWG considered the Panel report, all public comments, and the comments of SACATM in preparing the final ICCVAM test method recommendations provided in this report. This report will be made available to the public and provided to U.S. Federal agencies for consideration, in accordance with the ICCVAM Authorization Action of 2000 (ICCVAM 2000). Agencies must respond to ICCVAM within 180 days after receiving an ICCVAM test method recommendation. These responses will be made available to the public on the NICEATM/ICCVAM website (http://iccvam.niehs.nih.gov) as they are received.

The efforts of the many individuals who contributed to the preparation, review and revision of this report are gratefully acknowledged. We greatly appreciate the careful preparation of the BRDs by ECVAM and their prompt response to requests for additional information. We especially recognize all of the Panel members for their thoughtful evaluations and generous contributions of time and effort. Special thanks are extended to Dr. Karen Brown for serving as the Panel Chair and to Drs. Jack Levin, Melvyn Lynn, Anthony Mire-Sluis, and Jon Richmond for their service as Evaluation Group Chairs. The efforts of the PWG were invaluable for assuring a meaningful and comprehensive review. We especially thank the Chair of the PWG, Dr. Richard McFarland (FDA, Center for Biologics Evaluation and Research) for his effective leadership. The efforts of the NICEATM staff and support contractor in preparing the BRD, organizing the Panel meeting, and preparing this final report are greatly appreciated. We acknowledge Drs. David Allen and Elizabeth Lipscomb, Catherine Sprankle, James Truax, and Doug Winters of Integrated Laboratory Systems, Inc., the NICEATM support contractor, for their assistance. We also thank Dr. Raymond Tice, Deputy Director of NICEATM, for his efforts on this project.

This comprehensive ICCVAM evaluation of the validation status of these five test methods and the accompanying recommendations should aid agencies in providing guidance on their future use for regulatory safety testing. The ICCVAM recommendations for future studies are expected to advance broader applicability of these methods, which may further reduce animal use while ensuring continued or better protection of human health.

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