1.0 Introduction

In June 2005, the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) initiated a review of the validation status of five *in vitro* pyrogen test methods proposed as replacements for the rabbit pyrogen test (RPT). The test methods were submitted by 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. This submission was based on a validation study financed by the European Commission within the 5th Framework Programme of Directorate General Research and was recently published (Hoffmann et al. 2005a; Schindler et al. 2006). The proposed 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 (Cryo) Human WB
- The Human WB/IL-6 *In Vitro* Pyrogen Test
- The Human Peripheral Blood Mononuclear Cell (PBMC)/IL-6 *In Vitro* Pyrogen Test
- The Monocytoid Cell Line Mono Mac 6 (MM6)/IL-6 *In Vitro* Pyrogen Test

For simplicity, the submitted studies are referred to collectively as the ECVAM validation study in this document.

ICCVAM, which is charged with coordinating the technical evaluations of new, revised, and alternative test methods with regulatory applicability (ICCVAM 2000), unanimously agreed that the five submitted *in vitro* test methods should have a high priority for evaluation. 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 these evaluations; Dr. Marlies Halder was the ECVAM liaison to the PWG. Following a NICEATM pre-screen evaluation of the comprehensive background review documents (BRDs) submitted by ECVAM, NICEATM, ICCVAM and the ICCVAM PWG requested additional information and clarification from ECVAM on a number of issues. In March 2006, in response to this request, ECVAM submitted revised BRDs and a list of responses to address these issues.

NICEATM, which administers ICCVAM and provides scientific support for ICCVAM activities, subsequently prepared a comprehensive draft BRD that provided information and data from the validation studies and scientific literature to enable a peer review of the validation status of each of the five *in vitro* test methods. A request for any other data and information on these test methods and for nominations to serve on an independent, scientific pyrogenicity review panel (Panel) was made through a 2005 *Federal Register* (*FR*) notice (Vol. 70, No. 241, pp. 74833-74834, December 16, 2005, available at http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/FR/FR_E5_7410.pdf), through the ICCVAM electronic mailing list, and through direct requests to over 100 stakeholders. Panel nominations were received, but no additional data or information was submitted in response to this request.

Announcement of a public Panel meeting to review the validation status of the five *in vitro* pyrogen test methods and availability of the ICCVAM BRD was made through a 2006 *FR* notice (Vol. 71, No. 238, pp. 74533-74534, December 12, 2006, available at http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/FR/FR_E6_21038.pdf). The draft BRD was made publicly available on the NICEATM/ICCVAM website (http://iccvam.niehs.nih.gov). Additional information provided by ECVAM in response to a request from Panel was appended to this BRD. All of the information provided to the Panel was also made publicly available. Comments from the public and scientific community are available on the NICEATM/ICCVAM website.

The adequacy of the data and information contained in the ICCVAM BRD to support the ICCVAM draft test method recommendations were discussed by the Panel in a public meeting on February 6, 2007 at the National Institutes of Health campus in Bethesda, MD. A report of the Panel's recommendations (see **Appendix A**; Panel Report, available at http://iccvam.niehs.nih.gov/docs/pyrogen/PrRevPanFinRpt.pdf) was made available for public comment on the NICEATM/ICCVAM website (see *FR* notice [Vol. 72, No. 89, pp. 26395-26396, May 9, 2007], available at http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/FR/FR_E7_8896.pdf).

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

ICCVAM and the PWG then considered the Panel report, all public comments, and the comments of SACATM in preparing the final BRD and the final test method recommendations that are provided in this ICCVAM Test Method Evaluation Report. This report will be made available to the public and provided to U.S. Federal agencies for consideration (ICCVAM 2000). The ICCVAM final BRD, revised in response to the Panel and PWG comments, will also be provided as background information and technical support for this report. Agencies with applicable testing regulations and guidelines (see **Appendix B**) are required by law to respond to ICCVAM within 180 days of 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.