Preface

Endotoxin, a bacterial pyrogen also known as lipopolysaccharide, is an integral component of the Gram-negative bacterial cell membrane. Endotoxin directly interacts with host monocytoid cells to induce the release of a variety of proinflammatory cytokines (e.g., interleukin [IL]-1β, IL-6, tumor necrosis factor-α). In addition to an initial febrile reaction, excessive release of these cytokines during Gram-negative bacterial sepsis can lead to multiple organ failure and death. For this reason, it is critical that parenteral pharmaceuticals, fluids for injection, medical devices, and human biological products be properly and accurately evaluated for the presence of endotoxin prior to their clinical or veterinary use. The original pyrogen test, the rabbit pyrogen test (RPT), was developed in 1941 to limit to an acceptable level the risks of febrile reaction in the patient following administration of, or contact with, the product of concern. While the RPT continues to serve this purpose well, an endotoxin test using a hemolymph extract (i.e., "blood") from the horseshoe crab (i.e., the bacterial endotoxin test [BET]) was developed in the early 1970's as an *in vitro* alternative to the RPT for the detection of Gram-negative endotoxin. In 1980, the United States (U.S.) Food and Drug Administration (FDA) published guidelines for use of the BET as an end-product test for human and animal drug products. The U.S., European, and Japanese Pharmacopeias currently recognize both test methods for pyrogen testing (i.e., RPT and BET). The BET is recognized for its sensitivity to the presence of endotoxins from Gram-negative bacteria, but it also has some limitations, including its inability to respond to non-endotoxin pyrogens, as well as its susceptibility to interference from certain types of materials (e.g., products with high protein and lipid levels, glucans). In contrast, the RPT is capable of detecting both endotoxin and non-endotoxin pyrogens.

More recent efforts have focused on the development of *in vitro* test systems that might achieve or exceed the sensitivity of the BET and the RPT. Test systems based on the activation of human monocytes *in vitro* have been developed that take advantage of the role of these cells in the fever response. 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, conducted a validation study to independently evaluate the usefulness of six *in vitro* pyrogen test methods. The study was financed by the European Commission within the 5th Framework Programme of Directorate General Research and was recently published (Hoffmann et al. 2005a). Since two tests based on the acute monocyte leukemia cell line THP-1 did not meet the validation criteria, they are not included in the peer review. In 2004, the University of Konstanz (Germany) carried out catch-up validation studies of two tests using Cryopreserved whole blood (Cryo WB/IL-1β) or blood cells (cryopreserved or fresh peripheral blood mononuclear cells [PBMC]/IL-6), the results of which were recently published (Schindler et al. 2006).

Based on these studies, in June 2005, ECVAM submitted background review documents (BRDs) for five of these test methods, which were proposed as replacements for the RPT, to the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM). The five test methods are:

- The Human Whole Blood (WB)/IL-1β *In Vitro* Pyrogen Test

- The Human WB/IL-1β *In Vitro* Pyrogen Test: Application of Cryo Human WB
- The Human WB/IL-6 *In Vitro* Pyrogen Test
- The Human PBMC/IL-6 *In Vitro* Pyrogen Test
- The Monocytoid Cell Line Mono Mac 6/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 Authorization Act of 2000, [42 U.S. Code 285*l*-3, available at http://iccvam.niehs.nih.gov/docs/about_docs/PL106545.htm]), 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 Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) to carry out these evaluations. The PWG consists of knowledgeable scientists from ICCVAM member agencies. The PWG functions included reviewing draft test method BRDs, recommending proposed performance standards, identifying and recommending scientists for independent peer review panels, preparing questions for expert or peer review Panels, developing ICCVAM draft test method recommendations regarding the usefulness and applicability of the alternative test methods for regulatory testing, and recommending necessary future validation studies. ICCVAM and NICEATM also collaborate closely with ECVAM. Accordingly, an ECVAM liaison was designated for the ICCVAM PWG to provide additional clarification and information during the evaluation and review process.

NICEATM, which administers the ICCVAM and provides scientific support for ICCVAM activities, subsequently prepared a comprehensive draft BRD containing all of the information and data from the validation studies for each of the five *in vitro* test methods. A request for any other data and information on these test methods was made through a 2005 *Federal Register* (*FR*) request (Vol. 70, No. 241, pp. 74833-74834, December 16, 2005; available at http://ntp-apps.niehs.nih.gov/iccvampb/searchFR.cfm), through the ICCVAM electronic mailing list, and through direct requests to over 100 interested stakeholders. No additional data or information was submitted in response to this request.

The draft BRD was made publicly available on the NICEATM-ICCVAM website (http://iccvam.niehs.nih.gov). Comments from the public and scientific community were welcomed and were provided to the Panel and made available on the NICEATM-ICCVAM website (see *FR* notice [Vol. 71, No. 238, pp. 74533-74534, December 12, 2006], available at http://iccvam.niehs.nih.gov).

The independent review of the usefulness and limitations of the five test methods took place in a public meeting of the independent peer review panel (Panel) on February 6, 2007 at the National Institutes of Health in Bethesda, Maryland. The Panel considered the information and data available in the draft BRD. The Panel's independent peer review report was then 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). Following the Panel meeting, ICCVAM and the PWG considered the Panel's report and

public comments, and prepared this final BRD. ICCVAM and the PWG also considered the Panel's report, comments from the public and from the Scientific Advisory Committee on Alternative Toxicological Methods, and information in this BRD, and prepared final test method recommendations that will be provided to U.S. Federal agencies and made available to the public. These final recommendations are included in the ICCVAM Test Method Evaluation Report, which is available at http://iccvam.niehs.nih.gov/methods/pyrogen/pyrogen.htm, in accordance with the ICCVAM Authorization Act of 2000.

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