PREFACE

The Food Quality Protection Act and Amendments to the Safe Drinking Water Act in 1996 directed the U.S. Environmental Protection Agency (U.S. EPA) to develop and validate a screening program to determine whether certain substances may have hormonal effects in humans. In response, the U.S. EPA developed an Endocrine Disruptor Screening Program (EDSP), and is currently evaluating the scientific validity of screening and testing methods proposed for incorporation into the EDSP. *In vitro* estrogen receptor (ER) and androgen receptor (AR) assays have been proposed as possible components of the EDSP Tier 1 screening battery. The U.S. EPA asked the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) to evaluate the validation status of these *in vitro* assays. ICCVAM, which is charged with coordinating the technical evaluations of new, revised, and alternative test methods, agreed to evaluate the assays based on their potential interagency applicability and public health significance.

In order to assess the current validation status of these *in vitro* methods, it was first necessary to compile all of the available data and information for existing assays. The National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), which provides operational support for the ICCVAM, subsequently arranged for preparation of this Background Review Document (BRD) by its support contractor, Integrated Laboratory Systems, Inc. (ILS) with financial support from the U.S. EPA. This BRD reviews available data and procedures for existing *in vitro* AR transcriptional activation (TA) assays and is organized according to published guidelines for submission of test methods to ICCVAM (ICCVAM, 1999). Separate BRDs have also been prepared for *in vitro* ER binding assays, *in vitro* AR binding assays, and *in vitro* ER TA assays.

As part of the ICCVAM evaluation, the U.S. EPA also asked for development of minimum performance criteria that could be used to define an acceptable *in vitro* AR TA assay. It was envisioned that these criteria would be based on the performance of existing standardized *in vitro* AR TA assays. The minimum performance criteria could then be used to assess the acceptability of new or revised assays proposed in the future. However, a comprehensive review determined that there were no standardized *in vitro* AR TA assays with adequate validation data that could serve as the basis for establishing these performance criteria. An independent Expert Panel

(Panel) was therefore convened to assess the status of existing *in vitro* AR TA assays and to develop recommendations for standardized assays and validation studies that should be conducted. After adequate validation studies have been completed on one or more standardized AR TA assays, an independent Peer Review Panel will be convened to evaluate the validated assay(s) and to recommend minimum performance criteria for *in vitro* AR TA assays.

This BRD reviews available *in vitro* AR TA assays and presents the data available for substances evaluated in these assays. The relative performance of various types of *in vitro* AR TA assays is compared using this existing data, which was very limited for some of the assays. Based on the comparative performance and advantages and disadvantages of each type of assay, several assays are proposed as priority candidates for standardization and future validation. In addition, minimum procedural standards that should be used for *in vitro* AR TA assays are proposed. These standards include elements such as dose selection criteria, minimum number of replicates, appropriate positive and negative controls, criteria for an acceptable test run, and proficiency standards for participating laboratories. Finally, the BRD proposes a list of substances recommended for the validation of *in vitro* AR TA screening assays.

An Expert Panel was convened in a public meeting on May 21-22, 2002, to review the information and proposals provided in this BRD, and to develop conclusions and recommendations on the following:

- Specific assays that should undergo further evaluation in validation studies, and their relative priority for evaluation.
- The adequacy of proposed minimum procedural standards.
- The adequacy of protocols for specific assays recommended for validation studies.
- The adequacy and appropriateness of substances proposed for validation studies.

The Expert Panel meeting was announced to the public in a *Federal Register* notice (Vol. 67, No. 66, pp. 16415-16416, Apr. 5, 2002; also available on the internet at: http://iccvam.niehs.nih.gov/docs/FR/6716415.pdf)

An ICCVAM Endocrine Disruptor Working Group (EDWG) was organized to coordinate the technical evaluation of *in vitro* endocrine disruptor screening methods. The EDWG is co-chaired

by Drs. David Hattan and Marilyn Wind, and consists of knowledgeable scientists from ICCVAM agencies. The EDWG functions include identification and recommendation of experts for the Expert and Peer Review Panels, the review of test method BRDs for completeness, preparation of questions for the Expert and Peer Review Panels, and development of draft ICCVAM test recommendations based on Panel evaluations. Final ICCVAM test recommendations will be forwarded from the ICCVAM to Federal agencies for their consideration.

In July 2002, the draft of this BRD was revised to address corrections and omissions noted by the Expert Panel and published as a final version. The final report of the Expert Panel and a proposed list of substances for validation studies of *in vitro* ER and AR methods was published and made available to the public for comment as announced in a *Federal Register* notice (Vol. 67, No. 204, pp. 64902-64903, October 22, 2002; available at http://iccvam.niehs.nih.gov/docs/FR/6764902.htm). A final ICCVAM Test Method Evaluation report will be published in early 2003. This report will include ICCVAM recommendations, the final Expert Panel report, a recommended list of substances for validation studies, and public comments. The report will be forwarded to federal agencies for their consideration and made available to the public.

The efforts of the many individuals who contributed to the preparation, review, and revision of this BRD are gratefully acknowledged. These include Barbara Shane, Christina Inhof, Errol Zeiger, Raymond Tice, Bradley Blackard, Steven Myers, and Linda Litchfield, from ILS, Inc. who prepared the BRD. The suggestions and advice from the ICCVAM EDWG members and co-chairs on early drafts and subsequent versions were invaluable, as were the comments from *ad hoc* reviewers on the final draft. Additional comments and suggestions for improvement of this and future test method documents are welcome at any time.

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July 31, 2002

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