Preface

There is evidence linking exposure to natural and man-made substances in the environment to adverse effects on the endocrine and reproductive systems of mammals, birds, reptiles, amphibians, and fish (EPA 1997; NAS 1999). In response to growing concerns about possible adverse health effects in humans exposed to such substances, the U.S. Congress enacted relevant provisions to safeguard public health in the Food Quality Protection Act (FQPA) of 1996 (Public Law [P.L.] 104-170) and the 1996 Amendments to the Safe Drinking Water Act (SDWA) (P.L. 104-182). These laws require the U.S. Environmental Protection Agency (EPA) to develop and validate a screening and testing program to identify substances with endocrine disrupting activity. The EPA subsequently proposed an Endocrine Disruptor Screening Program (EDSP) (EPA 1998) and began efforts to standardize and validate test methods for inclusion in the EDSP. Validation assesses whether test methods are sufficiently accurate and reproducible for their intended use.

In April 2000, the EPA asked the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) to evaluate the validation status of *in vitro* estrogen receptor (ER) and androgen receptor (AR) binding and transcriptional activation (TA) assays, which were proposed as possible components of the EDSP Tier 1 screening battery. ICCVAM, which is charged by law (P.L. 106-545) to evaluate the scientific validity of new, revised, and alternative test methods proposed for specific regulatory uses, agreed to evaluate these assays based on their potential interagency applicability and public health significance. Because a large number of *in vitro* ER- and AR-based assays were known to exist, it was expected that at least some of these would have been adequately validated and could be rapidly included in the EDSP following a review of existing data and verification of their validity. The EPA also asked for the development of minimum performance standards that could be used to define acceptable *in vitro* ER and AR binding and TA assays. It was envisioned that these standards would be based on the performance of validated *in vitro* ER- and AR-based assays.

The National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) subsequently compiled available relevant data and information on the *in vitro* ER and AR binding and TA assays. A draft Background Review Document (BRD) was organized for each of the four types of assays according to published guidelines for submission of test methods to ICCVAM (ICCVAM 1999). This comprehensive review revealed that there were no adequately validated *in vitro* ER- or AR-based assays, and therefore, no assays that could serve as the basis for establishing minimum performance standards. It was also discovered that there was little consistency among available protocols, and that no test method protocol was adequately detailed and standardized. Therefore, minimum procedural standards were proposed that should be incorporated in standardized protocols for each of the four types of assays. These minimum procedural standards include critical elements such as dose selection criteria, number of replicates per test, appropriate positive and negative controls, and criteria for an acceptable test. In addition, each BRD included a list of proposed substances that should be used for the validation of *in vitro* ER and AR binding and TA assays.

ICCVAM asked its Endocrine Disruptor Working Group (EDWG) to assist NICEATM with the technical evaluation of the four types of *in vitro* endocrine disruptor assays. The EDWG, which is comprised of knowledgeable scientists from participating ICCVAM agencies, was charged with:

- identifying and recommending scientists for the Expert Panel;
- reviewing the four draft BRDs for completeness and accuracy;
- developing questions for the Expert Panel to consider during their deliberations;
- developing draft ICCVAM recommendations based on the conclusions and recommendations of the Expert Panel.

On March 23, 2001, a *Federal Register (FR)* notice (66 *FR* 57: 16278-16279, March 23, 2001) requested data and nominations of expert scientists for an independent peer review evaluation of *in vitro* ER and AR binding and TA assays for endocrine disruptor screening. Data and nominations were also solicited from Federal agencies and national and international professional societies and organizations. An Expert Panel consisting of 24 scientists was selected based on advice from the EDWG. The expertise of the members included reproductive toxicology, androgen and/or estrogen receptor binding and transcriptional activation assays, validation of alternative *in vitro* methods, ecotoxicology, and biostatistics. The Expert Panel members were from the United States, the United Kingdom, Canada, Japan, and Denmark, and included scientists from industry, academia, and government.

The Expert Panel was charged with reviewing the information and recommendations provided in the four draft BRDs, and developing 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 the proposed minimum procedural standards;
- the adequacy of protocols for specific test methods recommended for validation;
- the adequacy and appropriateness of substances proposed for validation studies.

The Expert Panel members were assigned to one of four groups, each group with primary responsibility for one of the four types of assays being considered. In addition, each member of the Expert Panel was asked to evaluate and comment on the other three types of assays.

The Expert Panel meeting was announced to the public in a *FR* notice (67 *FR* 66: 16415-16416, April 5, 2002), which also included an announcement of the availability of the four draft BRDs and a request for public comments. The public comments and information submitted in response to this notice were provided to the Expert Panel and the public in advance of the meeting. The Expert Panel met in public session on May 21-22, 2002, in Research Triangle Park, North Carolina. The Expert Panel presented the evaluations, conclusions, and recommendations for each of the four types of *in vitro* ER- and AR-based assays. Opportunities for public comment were provided during the meeting. After consideration of the public comments, the Expert Panel reached consensus on each of its recommendations. The Expert Panel's written evaluations and recommendations were consolidated into an independent report, which is included in this document as **Appendix A**.

Following the Expert Panel meeting, the EDWG, in collaboration with NICEATM, revised the draft minimum procedural standards and the draft list of proposed substances to incorporate the recommendations of the Expert Panel. The

four draft BRDs were subsequently revised to address corrections and omissions noted by the Expert Panel and published as final versions. Due to the length of these documents, they are not included in this report but are available on the ICCVAM/NICEATM website *http://iccvam.niehs.nih.gov/methods/endocrine.htm*.

In October 2002, the final report of the Expert Panel and the EDWG's revised list of proposed substances for validation of *in vitro* ER and AR binding and TA assays were made available to the public for comment (67 *FR* 204: 64902-64903, October 22, 2002). Following review of the public comments, the EDWG and ICCVAM finalized the recommendations that are provided in this report. These recommendations include suggested assays for future validation, minimum procedural standards, and substances that should be used to standardize and validate *in vitro* ER and AR binding and TA assays. The final Expert Panel report, public comments, and other relevant documents are appended to this document, all of which are available on the ICCVAM/NICEATM website *http://iccvam.niehs.nih.gov/methods/endocrine.htm*.

Use of the minimum procedural standards and the recommended validation substances should facilitate standardization and validation of *in vitro* endocrine disruptor assays, as well as facilitate test method comparison to determine which ones are the most sensitive and reliable. Data from studies to validate one or more test methods that incorporate the recommended minimum procedural standards will serve as the basis for developing minimum performance standards for acceptable *in vitro* ER- or AR-based assays. The EDSP will use data generated from validated *in vitro* and *in vivo* Tier 1 screening assays to make decisions, based on a weight-of-evidence approach, on whether to conduct large multi-generational *in vivo* studies. It is also anticipated that data obtained during the validation of the four different types of *in vitro* ER- and AR-based assays will help characterize the extent to which individual or batteries of *in vitro* endocrine disruptor assays might be used to prioritize chemicals for Tier 2 testing. Finally, implementation of the recommendations in this report are expected to decrease and perhaps eventually eliminate the need to use male and female animals as a source of AR and ER, respectively, for *in vitro* screening assays.

Since several Federal agencies are involved in supporting or conducting endocrine disruptor test method development and validation, or otherwise have an interest in endocrine disruptor testing, this report containing ICCVAM's recommendations will be forwarded to agencies for their consideration and information. Because the ICCVAM evaluation determined that none of these *in vitro* methods has been adequately validated, formal test recommendations will not be forwarded to Federal agencies. Following adequate validation and submission to ICCVAM of one or more of these *in vitro* endocrine disruptor methods, ICCVAM and NICEATM will coordinate their scientific peer review. After this review, formal ICCVAM test recommendations will then be forwarded to Federal agencies as required by the ICCVAM Authorization Act of 2000 (P.L. 106-545).

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