Evaluation Guidance to the Expert Panel for the Review of *In Vitro* AR/ER Binding Assays

A. General Instructions for the Expert Panel

The Panel is charged with reviewing the information and data provided in the Background Review Documents (BRDs) and developing conclusions and recommendations on the following:

1. *In vitro* AR/ER binding assays that should be considered for further evaluation in validation studies, and their relative priority for further evaluation.
2. The adequacy of the minimum procedural standards recommended for *in vitro* AR/ER binding assays.
3. The adequacy of available *in vitro* AR/ER binding test method protocols for use in validation studies.
4. The adequacy and appropriateness of substances recommended for validation studies of *in vitro* AR/ER binding assays.

An outline of specific items to be addressed by the Panel is provided in **Section B** below. The Panel is charged with developing a written report that summarizes its recommendations and conclusions for each question.

All members of the Test Method Evaluation Group, including Secondary Reviewers (as outlined in the Panel Group spreadsheet), are asked to answer all four sets of Evaluation Guidance Questions and submit responses to the Question Leader (see Questions Leader assignments below). Panel Members are also welcome to respond to questions for the other two Test Methods where they are not a designated reviewer. The Question Leader is responsible for compiling comments and developing a draft response for their question. The Breakout Group Chair is responsible for compiling each question's draft response into an overall draft position for the Breakout Group. This draft position will be circulated to each member of the Panel before the May review meeting for comment. The revised draft position will be presented and discussed at the Expert Panel review meeting in May.

Proposed Evaluation Guidance Question Leaders

***In Vitro* ER Binding BRD:**

Chair:	George Daston
Question 1:	Nira Ben-Jonathan
Question 2:	Bob Combes and James Wittliff
Question 3:	John Giesy and John Harbell
Question 4:	Stephen Safe
Statistician:	Walter Piegorsch

***In Vitro* ER Transcriptional Activation BRD:**

Chair:	John Stegeman
Question 1:	Grantley Charles
Question 2:	Ellen Mihaich and Tim Zacharewski
Question 3:	Tom Wiese
Question 4:	James Yager

Statistician: Shyamal Peddada

***In Vitro* AR Binding BRD:**

Chair: Terry Brown
Question 1: Thomas Gasiewicz
Question 2: Anne Marie Vinggaard
Question 3: Bernard Robaire
Question 4: Tohru Inoue
Statistician: Walter Piegorsch

***In Vitro* AR Transcriptional Activation BRD:**

Chair: Elizabeth Wilson
Question 1: William Kelce
Question 2: William Kelce
Question 3: Kevin Gaido
Question 4: Elizabeth Wilson
Statistician: Shyamal Peddada

B. Questions for Evaluating the *In Vitro* AR/ER Binding BRDs

1. *In Vitro* AR/ER Binding Assays: Recommendations and Priority for Validation Studies

1.1 The respective BRDs review the comparative performance, reliability, advantages, and disadvantages for different ***in vitro*** AR/ER binding assays, and recommend a relative priority for further development and/or validation based on this information (**Section 6.0)**. Considering that the intended use of the assays are as a toxicological screen, is the Panel aware of other advantages and disadvantages for the assays discussed in the BRDs?

1.2 Considering that the intended use of the assays are as a toxicological screen, does the Panel agree with the relative priority recommended for these sets of assays? Does the Panel recommend any changes in priority, or have specific recommendations for prioritization? In considering prioritization,

1.2.1 Are rat uterine cytosol and rat prostate cytosol the best sources of estrogen receptors and androgen receptors, respectively, for the binding assays?

1.2.2 Should the binding of compounds to different receptor isoforms be addressed in the binding assays?

1.2.3 Should a metabolic activation system be included in the binding assays?

2. Minimum Procedural Standards for *In Vitro* AR/ER Binding Assays

2.1 To facilitate assay standardization, the BRDs propose minimum procedural standards that should be incorporated into *in vitro* AR/ER binding assay protocols (**Section 12.2**). Considering that the intended use of the assays are as a toxicological screen, does the Panel agree with the adequacy of the proposed procedural standards? If not, what changes should be made to each standard and why?

2.1.1 Binding Constant (K_d) of the Reference Androgen/Estrogen

2.1.2 Reference Androgen/Estrogen
Should the reference androgen be an endogenous one rather than a synthetic androgen like R1881? In AR binding assays containing the progesterone receptor (PR) in addition to the AR, triamcinolone acetate is added to prevent the binding of R1881 to the receptor without interfering with the binding of either R1881 or test substances to the AR. Is enough known to predict that triamcinolone acetonide will not interfere with future test substances if this compound is routinely used in the assay?
2.1.3 Preparation of Test Substances
2.1.4 Concentration Range of Test Substances
2.1.5 Solvent and Positive Controls
2.1.6 Within Test Replicates
2.1.7 Dose Spacing
2.1.8 Data Analysis
2.1.9 Assay Acceptance Criteria
2.1.10 Evaluation and Interpretation of Results
2.1.11 Test Report
2.1.12 Replicate Studies

2.2 Considering that the intended use of the assays are as a toxicological screen, are there other minimum procedural standards that should be included? If so, what are they and why?

3. Recommendations for *In Vitro* AR/ER Binding Test Method Protocols for Validation Studies

3.1 A standardized *in vitro* AR binding assay protocol using rat prostate cytosol (RPC) and a standardized *in vitro* ER binding assay protocol using rat uterine cytosol (RUC) are provided in **Appendix B** of their respective BRDs. These two assays are proposed for validation studies by the U.S. EPA and other sponsors. **Section 12.3** discusses additional details that should be added, based on the minimum procedural standards in **Section 12.2**. In addition, an example of an *in vitro* ER Binding RUC assay (based on the U.S. EPA protocol), which incorporates the recommended minimum procedural standards is provided in **Section 12 Annex** of the **"***In Vitro* ER Binding BRD". Considering that the intended use of the assays are as a toxicological screen, would the current protocols, with the additions detailed in **Section 12.2** and **12.3**, provide a level of detail to appropriately minimize interlaboratory variability? If not, what revisions or additions should be made to the protocols?

3.2 In addition to the minimum procedural details listed in **Section 12.2,** are there other protocol elements that should be considered for other *in vitro* AR/ER binding assays recommended for validation as a toxicological screen, including those protocols provided in **Appendix B**?

3.3 Considering that the intended use of the assays are as a toxicological screen, is the Panel aware of other available standardized protocols for assays recommended for validation?

4. Recommended List of Substances to be Used for Validation of *In Vitro* AR/ER Binding Assays

4.1 **Section 12.4** provides a list of substances recommended for use in validation studies of *in vitro* AR/ER binding assays**.** Considering that the intended use of the assays are as a toxicological screen, does the Panel agree with the selection criteria, adequacy and appropriateness of substances recommended for validation studies, in terms of the following issues? If not, what substances should be added or deleted?

4.1.1 The number and distribution of substances across the range of measurable AR/ER binding activity, including negatives.

4.1.2 The number and range of substances by chemical class.

4.1.3 The number and range of substances by product class.