

## Evaluation Guidance to the Expert Panel for the Review of *In Vitro* AR/ER Transcriptional Activation (TA) 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 TA 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 TA assays.
3. The adequacy of available *in vitro* AR/ER TA test method protocols for use in validation studies.
4. The adequacy and appropriateness of substances recommended for validation studies of *in vitro* AR/ER TA 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:	Steve Safe
Statistician:	Walter Piegorsch

#### ***In Vitro* ER TA 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 TA 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 TA BRDs**

**1. *In Vitro* AR/ER TA 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 TA 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 What receptor types (species, isoform) are the best for the transcriptional activation assays?
  - 1.2.2 Should preference be given to cells with endogenous ER, transiently transfected ER expression vectors, or stably transfected ER expression vectors?
  - 1.2.3 Which response elements (species, sequence) are the best for the reporter vectors?

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

- 2.1 To facilitate assay standardization, the BRDs propose minimum procedural standards that should be incorporated into *in vitro* AR/ER TA 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 Transcriptional Activation of the Reference Androgen/Estrogen
- 2.1.2 Reference Androgen/Estrogen
- 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 TA Test Method Protocols for Validation Studies

3.1 Protocols provided by scientists with expertise in *in vitro* AR/ER TA test methods are provided in **Appendix B** of the respective BRDs. **Section 12.3** discusses additional details that should be added, based on the minimum procedural standards in **Section 12.2**. 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 standards listed in **Section 12.2**, are there other protocol elements that should be considered for *in vitro* AR/ER TA 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 TA Assays

4.1 **Section 12.4** provides a list of substances recommended for use in validation studies of *in vitro* AR/ER TA 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 transcriptional 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.