Test Guideline Paragraph (¶)	Comments
General	In general ICCVAM agrees with the OECD Peer Review Panel (PRP) comments on the draft Test Guideline (TG) for the Stably Transfected Transcriptional Activation (STTA) Assay for Detecting Estrogenic Activity of Chemicals. In particular, ICCVAM agrees that the draft TG is incomplete and needs considerable revision, especially regarding the lack of a clear definition for a positive response and insufficient guidance on the criteria for acceptable test performance. Because of these deficiencies, coupled with the numerous technical issues identified below, ICCVAM cannot agree with the PRP's conclusion that the test guideline is sufficiently detailed to permit others to perform the assay.
We concur with the technical comments and suggested modifications (Appendix 1) provided in the PRP Summary Report with particular emphasis on the following issues:	
¶ 1	It should be clearly stated that the STTA assay, as currently described in the TG, has been validated only for the detection of estrogen receptor (ER) agonist activity.
¶ 9	This paragraph should include considerably more detail regarding the establishment and maintenance of a historical database for reference standards and controls and how this information will be used to generate test acceptance criteria. Acceptance criteria should include: • a minimum fold-induction for the reference estrogen • a response that falls within an acceptable range for the positive and solvent controls • EC50 values for the E2 reference standards based on the historical database. Acceptance criteria should also include procedures for monitoring "edging effects." In addition, detailed guidance should be included concerning qualification of materials used to conduct the assay (plastic ware, cell culture media, reagents etc.)
¶ 11	More detailed information regarding the maintenance of the cell line should be included. At a minimum, the information should include the suggestions made by the PRP (i.e., guidance on the effect of passage number on response, method of monitoring stability of cell line, media volume requirements for testing).
¶ 14	The specific concentration of DMSO to be used as concurrent vehicle control should be stated (i.e., 0.1% v/v).

Test Guideline Paragraph (¶)	Comments
¶ 16	A Level 2 *in vitro* screening assay for the testing and assessment of endocrine disrupting chemicals in the OECD Conceptual Framework should be optimized to detect weakly acting ER agonists, therefore the TG should specify a limit dose of 1 mM, unless precluded by solubility or cytotoxicity constraints, as recommended in ICCVAM (2003)[1]. This recommended limit dose is based on EC_{50} values that are near or greater than 10 µM for a number of ER agonists tested in several different ER TA assays (ICCVAM 2002)[2], therefore, certain weak acting ER agonists may not be detectable if the 10 µM limit dose, as specified in the current draft TG, is used. Although DMSO and 1 nM E2 are the recommended vehicle and positive controls, respectively on the plate layout, there is no explanation for the 7-point E2 log dilution in columns 10-12. The presumption is that it will provide a full dose response curve for the reference estrogen, but there is no information in the TG regarding how this dose response will be used (e.g., Is it to be used as a quality control for acceptance testing? Is it to be used in conjunction with the positive control for calculations of PC50, PC10 or EC50?).
¶ 17	A weak acting ER agonist with a maximal TA response two to three orders of magnitude lower than the E2 reference estrogen (e.g., methoxychlor) should be included in triplicate on each plate as an additional positive control. The inclusion of a second positive control in addition to E2 would provide another quality control measure by which to judge the sensitivity and acceptability of the test method for detecting weak agonists. Considering that the current test plate layout includes nine wells for the E2 positive control, replacing three E2 wells with a weak acting agonist control would seem inconsequential.
¶ 20	This paragraph should define how the activity of the positive control is calculated and how this calculation relates to the data obtained from the E2 reference standard curve. As emphasized by the PRP, the TG should clearly define what constitutes a positive or negative response. The TG specifies the calculation of PC50 and PC10 but does not indicate how either calculation is used for other than as noted in paragraph 23 ("in general, when PC50 can be calculated, a test chemical is considered positive"). The TG also states that an EC50 should be calculated using a Hill equation when dose response data is applicable but does not indicate how this information is to be used in conjunction with PC50 or PC10 values. Although not as critical in the testing of estrogen agonists, there is no clear guidance for monitoring cell viability and how cytotoxic concentrations of test substances may affect results. The TG should also briefly discuss how the information derived from the assay is to be used within the OECD Conceptual Framework for the Testing and Assessment of Endocrine Disrupting Chemicals.

Test Guideline Paragraph (¶)	Comments
Editorial	In the title of the draft TG, "Activation" is misspelled.

[1]ICCVAM Evaluation of the *In Vitro* Methods for Detecting Potential Endocrine Disruptors: Estrogen Receptor and Androgen Receptor Binding and Transcriptional Activation Assays (NIH No: 03-4503. Available: http://iccvam.niehs.nih.gov/methods/endocrine/endocrine.htm).
[2]ICCVAM Background Review Document: Current Status of Test Methods for Detecting Endocrine Disruptors, *In Vitro* Methods for Detecting Estrogen Receptor Transcriptional Activation Assays (NIH No: 03-4505. Available: http://iccvam.niehs.nih.gov/methods/endocrine/endocrine.htm).