Primary Reviewer: _____ **Signature:** _____
[Insert Name of Organization] **Date:** _____
Secondary Reviewer: _____ **Signature:** _____
[Insert Name of Organization] **Date:** _____

Template version 08/2011

DATA EVALUATION RECORD

STUDY TYPE: Estrogen Receptor Transcriptional Activation (Human cell Line, HeLa-9903); OCSPP 890.1300; OECD 455.

PC CODE: *(if applicable)* **DP BARCODE:** *(if applicable)*

TXR#: *(if applicable)* **CAS No.:** [#]

TEST MATERIAL (PURITY): *(use name of material tested as referred to in the study (common agency chemical name in parenthesis))*

SYNONYMS: *(Other names and codes)*

CITATION: Author *(up to 3, see SOP for exact format)*. ([Study Year]). Title. Laboratory name and location. Laboratory report number, study completion date. MRID *(if applicable) (no hyphen)*. Unpublished. *(OR if published, list Journal name, vol.:pages)*

SPONSOR: *(Name of Study Sponsor)*

EXECUTIVE SUMMARY: In an estrogen receptor transcriptional activation assay (MRID (if applicable) [number]) conducted by [lab], [cell type] cells cultured *in vitro* were exposed to [chemical (%., batch/lot #)] at concentrations of [#, #, #, and #] mg/mL in [solvent (final concentration of solvent)] for [provide duration of exposure]. The experiments were performed using [96]-well plates and each [chemical] concentration was tested in triplicate (3 wells/plate). Cells were exposed to the test agent for [23 ±1] hr to induce reporter (luciferase) gene products. Luciferase expression in response to activation of the estrogen receptor by [chemical] was measured upon addition of a luciferase substrate and detection with a luminometer with acceptable sensitivity.

[Chemical] was tested up to [cytotoxic/insoluble/limit concentrations (*e.g.*, 1 µl/mL, 1 mg/mL or 1 mM), *include other details as appropriate*]. The positive control and reference chemicals [did/did not] induce the appropriate responses. The RPC_{max} (maximum level of response induced by chemical expressed a percentage of the response induced by 1 nM estradiol (E2) on the same plate) was [#]. The PC_{max} was [#]. *Report whether or not the test material was positive for estrogen receptor transcriptional activation and if so, report the PC_{10} (and, if applicable, the PC_{50}) for each run.*

This study [satisfies/does not satisfy] the Test Order requirement for an Estrogen Receptor Transcriptional Activation assay (OCSPP 890.1300; OECD 455).

(If it does not satisfy the requirement, concisely list only the major deficiencies and refer to deficiency section.)

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements [were/were not] provided. *(Discuss deviations from regulatory requirements)*

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Substance: *Common name as used by Agency*

Description: *e.g. technical, nature, color, molecular weight*
Source: *include catalog #*
Lot/Batch #: *include expiration date*
Purity: [#]%
Solubility:
Volatility:
Stability:
Storage conditions:
Vapor pressure: *if applicable (a highly volatile substance may have an effect on adjacent cells and require the use of plate sealers)*
CAS #: *CAS # or* Not available
Structure: *[Structure] or* Not available

2. Reference substances

17β-estradiol (strong estrogen; positive control)
Supplier: *Source/company (City, State [and Country, if outside U.S.A.])*
Catalogue and Batch #:
Purity:
CAS # : 50-28-2

17α-estradiol (weak estrogen)
Supplier: *Source/company (City, State [and Country, if outside U.S.A.])*
Catalogue and Batch #:
Purity:
CAS # : 57-91-0

Corticosterone (negative compound)
Supplier: *Source/company (City, State [and Country, if outside U.S.A.])*
Catalogue and Batch #:
Purity:
CAS # : 50-22-6

17α-methyltestosterone (very weak agonist)
Supplier: *Source/company (City, State [and Country, if outside U.S.A.])*
Catalogue and Batch #:
Purity:
CAS # : 58-18-4

3. Vehicle(s)

Solvent: (supplier and lot, usually water, ethanol [report purity/%] or DMSO)
Solvent control (final concentration): (DMSO should not exceed 0.1%)

B. METHODS

1. Cell Culture: *Example text follows. Alter as necessary to apply to specific procedures used by performing laboratory. Note any deviations from standard protocol and provide acceptable justification or report the deviations as deficiencies.*

Stably-transfected hERα-HeLa-9903 cells were obtained from the Japanese Collection of Research Bioresources (JCRB) Cell Bank and were verified to be free of mycoplasma infection using RT PCR. Cells were maintained in Eagles Minimum Essential Medium (EMEM) without phenol red, supplemented with kanamycin (60 mg/L) and 10% dextran-coated charcoal-treated fetal bovine serum (DCC-FBS; [source, in-house or commercial]; Lot[#]), in an incubator under 5% CO_2 at 37ºC. Upon reaching 75-90% confluency, cells were subcultured [#] times *(should be at least once but not more than 40)* prior to exposure to the test material. *(If the DCC-FBS was prepared at the performing laboratory, describe the protocol to ensure that all hormones have been adequately stripped from the media).*

2. **Transcriptional Activation Assays:** For each test, cells were plated in [*describe plate, noting plastic which is free of estrogenic activity*] at a density of [*should be* $1x10^4$ cells/100 µL medium/well in a 96 well plate] and allowed to attach for [#] hours. The Growth media was replaced with media containing serial log dilutions of [chemical] in [solvent vehicle]. Cells were incubated for [20-24 hours] at 37±1°C. The total final concentration of [vehicle] was [#%]. Cytotoxicity was determined by [method]. Due to the volatility of the chemical, a plate sealer was used to isolate individual wells during testing (*delete this sentence if the chemical is not volatile*).

   Transcriptional activation of the estrogen receptor was determined using Steady-Glo® Luciferase Assay System (Promega, Madison, WI). *(Describe the commercial luciferase assay reagent or system used to determine luciferase gene activation. Describe luminometer and sensitivity).*

a. **Preliminary Test:** A preliminary test evaluating concentrations ranging from [10^{-3} to 10^{-11} M] was conducted to determine the appropriate concentration range and to determine concentrations resulting in insolubility and/or cytotoxicity. *(Chemicals are to be tested up to a maximum concentration of 1 µl/mL, 1 mg/mL or 1 mM, whichever is lowest).*

b. **Proficiency Chemicals:** The responsiveness of the test system was confirmed for each newly prepared batch of cell stocks taken from the frozen stock by testing the following set of proficiency chemicals [in duplicate on separate days]. (*Include citation (e.g. MRID #) if applicable).*

Compound	CAS No.	Concentration Range (M)	Expected Response [a]	Notes
Diethylstilbestrol (DES)	56-53-1	10^{-14} to 10^{-8}	Positive	---
17α-Ethynyl estradiol (EE)	57-63-6	10^{-14} to 10^{-8}	Positive	---
Hexestrol	84-16-2	10^{-13} to 10^{-7}	Positive	---
Genistein	446-72-0	10^{-12} to 10^{-5}	Positive	Cytotoxic at 0.01[b], 0.1, and 1 mM
Estrone	53-16-7	10^{-12} to 10^{-6}	Positive	---
Butyl paraben	94-26-8	10^{-11} to 10^{-4}	Positive	Cytotoxic at 0.1[b] and 1 mM
1, 3, 5-Tris(4hydroxyphenyl)benzene [c]	15797-52-1	10^{-12} to 10^{-5}	Positive	Cytotoxic at 100µM. PC_{max} approx. 50% of PC. Binds to hERα and has ER antagonistic activity
Dibutyl phthalate (DBP)	84-74-2	10^{-11} to 10^{-4}	Negative [d]	Cytotoxic at 1 mM
Atrazine	1912-24-9	10^{-11} to 10^{-4}	Negative	Cytotoxic at 1 mM [b]
Corticosterone	50-22-6	10^{-10} to 10^{-4}	Negative	If not cytotoxic at 1 mM, then that should be the highest tested concentration

a Positive = $RPC_{max} \geq 10\%$ of the response of the positive control in at least 2 of 2 (or 2 of 3) runs
Negative = RPC_{max} fails to achieve at least 10% of the response of the positive control in 2 of 2 (or 2 of 3) runs
b Cytotoxicity is expected to be close to 80% at this concentration.
c Compound selected to challenge solubility and cytotoxicity
d DBP is negative for ERα mediated transcriptional activation, but may not be negative for non-ERβ mediated transcriptional activation. A positive result would indicate that the system is detecting activity other than that due to pure ERα, and is therefore unacceptable.

c. Reference Chemicals: To ensure the stability of the response from the cell line, six concentrations of each of the following reference chemicals were included in each plate in the current assay, along with the test chemical:

Reference Chemical	CAS No.	Concentration Range	Class
17β-estradiol	50-28-2	10^{-14} to 10^{-8}	Strong estrogen
17α-estradiol	57-91-0	10^{-12} to 10^{-6}	Weak estrogen
Corticosterone	50-22-6	10^{-10} to 10^{-4}	Negative compound
17α-methyltestosterone	58-18-4	10^{-11} to 10^{-5}	Very weak agonist

3. Data analysis: *List parameters that were analyzed and any statistical methods used. Include a statement that indicates whether the reviewers consider these analyses to be appropriate. If inappropriate, provide alternative/rationale. When software is used for data analysis, report the software title, version number, and source (company, city, state and country if outside U.S.). Report the method used to obtain the relative transcriptional activity compared to the positive control of 1 nM E2. Results from concentrations of the test material causing cytotoxicity (reducing the cell number by ≥20%) should be excluded from analyses. The following example text should be altered according to the specific data analysis procedures used by the performing laboratory:*

To obtain the relative transcriptional activity to the 1 nM E2 positive control (PC), the luminescence signals from the concurrent plate were analyzed by subtracting the mean value of the vehicle control from each well value to normalize the data; each normalized value was then divided by the mean value of the normalized PC. The resulting value was multiplied by 100 in order to express relative transcriptional activity as a percentage of the PC. Graph Pad Prism v. 5 (GraphPad Software, Inc., La Jolla, CA) was used to calculate the EC_{50}, PC_{10},

PC_{50}, RPC_{Max}, and PC_{Max} for [chemical] when applicable. The test material was defined as [positive/negative] for inducing estrogen receptor transcriptional activation if the RPC_{Max} [≥/<] PC_{10} in at least 2 of 2 (or 2 of 3) runs. $LogEC_{50}$ and Hill slop values are calculated only if a positive response is observed. Coefficients of variation (CV) were calculated for the luminescence data triplicates. Concentrations showing >20% cytotoxicity or evidence of insolubility were excluded from analyses.

4. Definitions

EC_{50} = concentration of agonist that induces a response halfway between the baseline (bottom) and maximum (top) response

PC_{10} = concentration of a test chemical at which the response is 10% of the response induced by the positive control (E2 at 1 nM) in each plate

PC_{50} = concentration of a test chemical at which the response is 50% of the response induced by the positive control (E2 at 1 nM) in each plate

RPC_{Max} = maximum level of response induced by a test chemical, expressed as a percentage of the response induced by the positive control (1 nM E2) on the same plate

PC_{Max} = concentration of a test chemical inducing the RPC_{Max}

II. RESULTS

A. PRELIMINARY TEST: *Include concentrations tested and the results regarding cytotoxicity, solubility limitations (e.g., precipitation, cloudiness) and the rationale for concentration selection for main study (Table 1).* Based on these results, concentrations of [#, #, #, #, #, #, and #] were selected for the assay.

Table 1. Preliminary Test for Solubility, Cytotoxicity, and Concentration-Selection for [Chemical][a]

Concentration (M)	% Viability [b]	Comments [c]
10^{-3}		
10^{-4}		
10^{-5}		
10^{-6}		
10^{-7}		
10^{-8}		
10^{-9}		
10^{-10}		
10^{-11}		
E2 1nM		
VC [d]		

a Data were obtained from page [#] of the study report.
b If viability is <80%, the concentration is considered cytotoxic.
c Include comments related to solubility issues, if applicable.
d VC = Vehicle control

B. Positive and Negative Reference Chemicals

1. **Proficiency Chemicals:** The responsiveness of cells to the required proficiency chemicals [was/was not] performed in [*at least duplicate on different days*]. The data are reported in Table 2. The responses [did/did not] demonstrate proficiency. *Report the results of this test in reference to the assay for the chemical of interest. Discuss any deviations from appropriate responses (positive, negative, and concentrations resulting in cytotoxicity).*

Table 2. Proficiency Chemicals [a]

Compound	Expected Response	Lab Response
Diethylstilbestrol	Positive	
17α-Ethynyl estradiol	Positive	
Hexestrol	Positive	
Genistein	Positive	
Estrone	Positive	
Butyl paraben	Positive	
1, 3, 5-Tris(4hydroxyphenyl)benzene	Positive	
Dibutyl phthalate	Negative	
Atrazine	Negative	
Corticosterone	Negative	

a Data were obtained from page [#] of the study report.

2. **Reference Chemicals:** Values derived from the concentration response curve (*e.g.*, log PC_{50}, log PC_{10}, log EC_{50}, Hill slope) for the four concurrently run reference materials are included in Table 3. *Compare these values to the performance criteria limits. Enter values for each of the two (or three) runs, and place an "X" in the appropriate column indicating whether or not the values for each parameter fell within the acceptable range for each reference chemical. State whether or not all of the reference chemicals performed within the criteria limits for each run.*

Table 3. Performance Criteria for Reference Chemicals[a]

Reference Chemical Parameter	Acceptable Range	Values			Acceptable	
		Run 1	Run 2	Run 3	Yes	No
17β-estradiol						
Log PC_{50}	-11.4 to -10.1					
Log PC_{10}	<-11					
Log EC_{50}	-11.3 to -10.1					
Hill Slope	0.7 to 1.5					
Test range	10^{-14} to 10^{-8} M					
17α-estradiol						
Log PC_{50}	-9.6 to -8.1					
Log PC_{10}	-10.7 to -9.3					
Log EC_{50}	-9.6 to -8.4					
Hill Slope	0.9 to 2.0					
Test range	10^{-12} to 10^{-6} M					
Corticosterone						
Test range	10^{-10} to 10^{-4} M					
17α-methyltestosterone						
Log PC_{50}	-6.0 to -5.1					
Log PC_{10}	-8.0 to -6.2					
Test range	10^{-11} to 10^{-5} M					

a Data were obtained from page [#] of the study report.

C. DEFINITIVE ASSAY

1. **Vehicle and Positive Controls:** Data for the vehicle and positive controls are included in Table 4. The overall mean TA value for the vehicle control was [#]. The mean normalized value for the positive control was [#]. The PC_{50} (50% of the maximum response) for E2 in this assay is [#] and the PC_{10} (10% of the maximum response) is [#].

Table 4. Transcriptional Activation (TA) Response of Vehicle and Positive Control [a]

Sample	Vehicle Control		Positive Control [b]		
Runs	Mean	SD	Mean	SD	Fold Induction [c]
1					
2					
3					

a Data were obtained from page [#] of the study report. *Include if 3 runs were conducted. Delete third column if only two runs were conducted.*
b Positive control was 17β-estradiol (E2) at 1 nM.
c Fold-induction = (mean TA of PC)/(mean TA of VC)

2. **Test Material:** *Based on the results of two (or three) independent runs, report the RPC_{max}, which is the maximum level of response induced by a test chemical, expressed as a percentage of the response induced by 1 nM E2 on the same plate, as well as the PC_{max}, the concentration associated with the RPC_{max}. For positive chemicals, report the concentrations that induce the PC_{10} and, if appropriate, the PC_{50}. The example text included below should be altered as necessary, depending on the data (e.g., a test chemical that is negative for inducing transcriptional activation would not have a PC_{10} or PC_{50}). Include a graph for each run (2 or 3 runs) as depicted below in Figure 1.*

Relative (to the PC) transcriptional activation at each concentration of the test chemical during the [2 or 3] assay runs is presented in Table 5, along with the mean of [both or all] of the runs. The concentration-response curves depicting fold induction of relative transcriptional activation is presented in Figure 1 (*Figure 2, and Figure 3 if applicable*) below. The mean RPC_{max} was [#]%, and the associated PC_{max} was [#] M. Because the $RPC_{Max} \geq PC_{10}$ in [both or two of the three] runs, [chemical] was considered positive for estrogen receptor transcriptional activation. The PC_{50} was [10^{-n}] M.

An example of a negative response may read as follows:

Because the $RPC_{Max} < PC_{10}$ in [two of the three or all] runs, [test material] was considered negative for estrogen receptor transcriptional activation in this test system.

Table 5. Relative Transcriptional Activation (RTA) of [chemical][a]

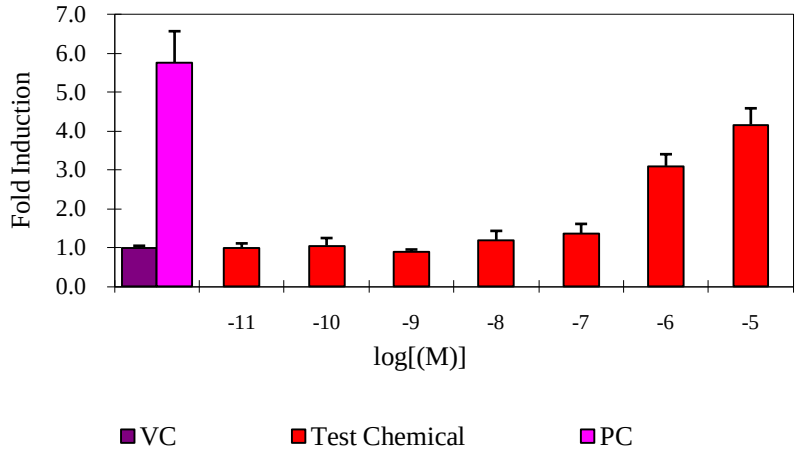
Parameter	RTA (mean ± SD); % of Positive Control (PC)					
	Run 1		Run 2		Run 3	
Conc. (M)	Mean	SD	Mean	SD	Mean	SD
[10^{-5}]						
[10^{-6}]						
[10^{-7}]						
[10^{-8}]						
[10^{-9}]						
[10^{-10}]						
[10^{-11}]						
Log EC_{50}[b]						
Hill Slope[b]						
RPC_{max}						
PC_{max}						
PC_{50}						
PC_{10}						

a Data were obtained from page [#] of the study report. *Include if 3 runs were conducted. Delete third column if only two runs were conducted.*

b Report if appropriate

NA= Not Applicable

Figure 1. Fold Induction of Relative Transcription Activation (RTA) of [chemical] compared to the Positive Control.



VC= Vehicle Control

PC= Positive Control (1 nM E2)

3. Performance Criteria: *Evaluate and discuss the results of the assay according the performance criteria, noting any deviations and the impact on the acceptability. Example text is included below.*

- Proficiency chemicals were tested in duplicate on different days, with appropriate responses (Table 2).
- Results of the 4 concurrent reference chemicals included in each experiment fell within the acceptable range (Table 3).
- Triplicate samples of the positive control (PC, 1 nM E2) and vehicle control (VC) were included in each plate.
- The mean luciferase activity of the positive control was at least 4-fold that of the mean vehicle control on each plate (Table 4).
- The fold-induction corresponding to PC_{10} of concurrent PC was greater than 1+2 standard deviations of the fold-induction value of the concurrent VC.
- The variability among raw data triplicates (*i.e.*, luminescence intensity data) was minimal (CV less than 20%), indicating a reliable PC_{10}.
- The results were reproducible among runs.

III. DISCUSSION AND CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: *Provide a brief paragraph of the investigators' conclusions.*

B. REVIEWER COMMENTS: *Discuss the RPC_{max}, as well as the associated PC_{max}. For positive chemicals, report the concentrations that induce the PC_{10} and, if appropriate, the PC_{50}. Discuss any discrepancy with investigators' conclusions; e.g. include rationale for acceptability or not; necessity for repeat. If unacceptable, is the study potentially upgradable to acceptable, and how?*

C. STUDY DEFICIENCIES: *List each deficiency (distinguishing between major and minor ones) and indicate what data is required to resolve the deficiency. If no data can be provided to satisfy the deficiency, indicate the need to repeat the assay.*