STEROIDOGENESIS (HUMAN CELL LINE – H295R)
OCSPP Guideline 890.1550

Standard Evaluation Procedure (SEP)

ENDOCRINE DISRUPTOR SCREENING PROGRAM

U.S. Environmental Protection Agency

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I. INTRODUCTION

A. Use of the Standard Evaluation Procedure

This Standard Evaluation Procedure (SEP) provides guidance on how to review studies conducted using the OCSPP Guideline 890.1550 Steroidogenesis (Human Cell Line – H295R) Assay that are submitted to support requirements imposed under the U.S. Environmental Protection Agency's Endocrine Disruptor Screening Program (EDSP). The product of the review will be a Data Evaluation Record (DER) that reflects how well the study conforms to the Guideline, and evaluates how well the study was performed, and provides the appropriate conclusions supported by the data. The DER will include, for example, a list of any significant deviations from the protocol as well as their potential impacts, a list of significant information missing from the study report, and any other information about the performance of the study that affects interpretation of the data within the context of the EDSP.

The DER should contain adequate information to provide the EPA with the ability to determine whether the study was performed according to the guideline. The objective of EDSP Tier 1 assays is to characterize the potential of a chemical to interact with the endocrine system.

The Guideline recommends the critical materials, methods, and analyses that lead to successful performance of the assay. If a particular material, method, or analysis is named in the Guideline, it is usually because other materials, methods, or analyses are either known to be inappropriate or at least have not been validated or that there is concern for their potential influence on results. The Agency has posted Corrections and Clarifications on Technical Aspects of the EDSP Tier 1 Assays (OCSPP Test Guideline Series 890) in the docket; the link to this document may be found by way of the EDSP web page (://www.epa.gov/endo/). It is therefore important to note deviations from specific materials, methods, or analyses in the DER, and provide the Agency's opinion on whether the deviation/deficiency has an impact on the performance and results of the study or the acceptability of the study.

II. STEROIDOGENESIS ASSAY

A. Purpose of the Assay

The human H295R adrenocortical carcinoma cell line has been shown to be a useful *in vitro* model for steroidogenic pathways and processes (Hecker *et al*., 2006; Hilscherova *et al*., 2004; Sanderson *et al*., 2002). The H295R cell line has physiological characteristics of zonally undifferentiated human fetal adrenal cells (Gazdar, *et al*., 1990). These cells represent a unique *in vitro* system in that they have the ability to produce the steroid hormones found in both the adult adrenal cortex and the gonads, allowing testing for effects on both corticosteroid synthesis and the production of sex steroid hormones, such as androgens and estrogens. Use of the H295R cell assay allows direct measurement of the cells hormone production (specifically testosterone and estradiol) and cell viability/cytotoxicity, which is important to discriminate between effects due to cytotoxicity or effects due to the direct interaction of a chemical with steroid hormone production. Additionally, use of an immortalized cell line eliminates the use of live animals for this purpose.

B. Study Design

After determining that cell line and assay parameters are adequate, test chemicals are evaluated utilizing a standardized protocol. The assay is performed under standard cell culture conditions in 24-well plates.[1] After an acclimation period of 24 hours, the cells are exposed to multiple concentrations of the test chemical in triplicate for 48 hours. In parallel, a Quality Control (QC) plate is run with known inhibitors and inducers of hormone production. At the end of the exposure period, the medium is removed from the wells, and the levels of hormones released by the cells are measured. Additionally, cell viability in each well is analyzed immediately after removal of the medium. Three independent runs of the assay should be conducted. For an individual run, normally six concentrations of the test chemical are tested in triplicate.

C. Pre-test Preparation

Prior to conducting any assay using the H295R cells, the Guidelines recommend taking certain steps (described below) to demonstrate that the cell line and assays are adequate. They also recommend that the laboratory demonstrate proficiency with running the assay.

1. Source of Cell Line and Passages

The Steroidogenesis Assay uses the human adrenocortical carcinoma H295R cell line, obtained from the American Type Culture Collection (ATCC CLR-2128). The propagation medium specified by ATCC for this cell line is a complete growth medium, consisting of DMEM:F12 medium supplemented with ITS+ Premix (insulin, transferrin, selenium, bovine serum albumin, and linoleic acid; final dilution 1:100) and Nu-Serum (final concentration 2.5% v/v).

Due to changes in the estradiol-producing capacities of the H295R cells with age (Hecker *et al.*, 2006), the cells are cultured following a specific protocol before they are used in experiments. Upon arrival from ATCC, the cells are grown for five passages, at which point they may be used or processed and stored frozen in liquid nitrogen. Cells started from frozen batches are cultured for a minimum of four additional passages prior to use in exposure experiments. To ensure adequate basal (estradiol) E2 production, the guideline recommends a maximum of 10 cell passages.

2. Hormone Measurement

Before testing unknown chemicals, a laboratory is expected to demonstrate the sensitivity of its hormone measurement system. Regardless of the hormone assays used, it is recommended that "chemical interference test" be performed to determine if the test chemical(s) will interfere with that hormone measurement system (see Section IV. A.1).

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[1] Alternatively, 48-well plates may be used for conducting the assay; however, seeding and experimental conditions should be adjusted accordingly.

3. Laboratory Proficiency Test

The Test Guideline recommends that the laboratory proficiency test in section IV. A. 2 be performed before testing unknown chemicals as demonstration of achieving and maintaining appropriate cell culture and test conditions for the successful conduct of the assay. As the performance of an assay is directly linked to the laboratory personnel conducting the assay, it is recommended that these procedures be repeated if a change in laboratory personnel occurs.

The proficiency test includes successfully demonstrating that the laboratory can characterize the effects of a known inducer (forskolin) and a known inhibitor (prochloraz) as described in section IV. A. 2.

It is also recommended that a quality control plate be run to evaluate the proficiency of a new batch of cells prior to use for chemical testing.

III. EVALUATION OF STUDY CONDUCT

A. Test Compound

It is recommended that the Steroidogenesis (H295R) Assay be performed with the technical (purest) form of the chemical intended for commercial use. The specifications of the test material should be clearly indicated in the study report and include the following: CAS Number, molecular formula, molecular weight, source, lot number, purity, storage conditions, and the identity of any contaminants present at concentrations ≥1%. Dimethyl Sulfoxide (DMSO), water, or ethanol are the most common solvents used in cell culture experiments. If another solvent is to be used, it is recommended that the registrant or laboratory justify the choice.

B. Cell Line and Culture Conditions

The cell line and medium conditions used in this assay are described in section II C.1. Where laboratories choose to deviate from the specified medium, EPA recommends that laboratories describe the deviation, the reason for the deviation, and demonstrate that the deviation does not alter the reliability or sensitivity of the assay. Additionally, EPA recommends that the Nu-Serum be tested for background concentrations of testosterone and estradiol, and the same batch of Nu-Serum must be used for each set of experiments. The culture atmosphere is 95% air, 5% CO_2 with a temperature of 37°C.

C. Dose Preparation, Plating and Pre-Incubation, and Exposure

1. Dose Preparation

EPA recommends that test chemicals and control chemicals (forskolin and prochlroaz) solutions be prepared as stock solutions and diluted according to the Test Guideline.

2. Plating and Pre-Incubation

Due to changes in the estradiol producing capacities of the cells with age (i.e., cell passage; Hecker *et al*., 2006), cells are cultured following a specific protocol before they are used for the

conduct of experiments. After initiation of an H295R culture from an original ATCC batch following the procedures outlined in the H295R Culture Protocol, cells are grown for five (5) passages (cells need to be split 4-times), and then frozen in liquid nitrogen. It is recommended in the Guideline that cells starting from these frozen batches need to be cultured for at least four (4) additional passages (passage # 4.5) before they can be used to conduct the assay. A total of ten passages is the maximum number that has been generally demonstrated to produce adequate basal E2 levels to perform the assay. Before using cells in a chemical exposure experiment, it is recommended that a QC plate be run to verify adequate basal production of testosterone and estradiol by the cells.

The number of cell culture dishes needed for an experiment depends on the number needed for the exposure experiment and the confluency of the cells in the culture dishes. A suggested rule is to use one 100 mm cell culture dish of 95-100% confluent cells to plate two 24-well plates at a target density of 200,000 to 300,000 cells/mL.

At 24 hours, the preferred density of cells in the wells for optimal hormone production in the medium is 50 – 60% confluent. At higher densities, cells tend to be affected by hormonal feedback or other mechanisms, and testosterone as well as E2 production patterns are altered. Consequently, EPA recommends that before conducting the assay the first time, different seeding densities between 200,000 and 300,000 cells per mL be tested, and the density resulting in 50 to 60% confluent growth in the well at 24 hours be selected for further experiments. The seeded plates are then incubated under the conditions previously described for 24 hours.

3. Exposure

The cells are checked microscopically for condition (attachment and morphology), and the medium is removed and replaced. The cells are then exposed by adding 1 µL of the appropriate stock solution in DMSO (or appropriate solvent), yielding a 1:1000 dilution. Dosing is in triplicate wells for each concentration. A suggested dosing schematic for the test chemical on the culture plate is presented in Table 1. The concentrations selected may be adjusted based on solubility limits or cytotoxicity. A concurrent QC plate is exposed as described in Table 5. The plates are then incubated as described above for 48 hours.

Table 1. Dosing schematic for test chemicals (final concentrations in µM).

	1	**2**	**3**	**4**	**5**	**6**
A	DMSO	DMSO	DMSO	0.1	0.1	0.1
B	100	100	100	0.01	0.01	0.01
C	10	10	10	0.001	0.001	0.001
D	1	1	1	0.0001	0.0001	0.0001

To terminate exposure, the medium is removed, stored for subsequent hormone analyses and cell viability is determined.

D. Cell Viability Measurements

A cell viability/cytotoxicity assay of choice can be used to determine the potential impact of the test chemical on cell viability. The Guideline calls for the assay to provide a true measure of

the percentage of viable cells present in a well, or that it be shown to be directly comparable to the Live/Dead® Cell Viability/Cytotoxicity Kit (Molecular Probes, Eugene, OR). An alternative assay that has been shown to work equally well as this kit is the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] test (Mosman *et al*., 1983). It should be noted that the assessment of cell viability using the above methods is a relative measurement that does not necessarily exhibit linear relationships with the absolute number of cells in a well. Therefore it is recommended that a subjective parallel visual assessment of each well be conducted, and digital pictures of the solvent controls and the two greatest non-cytotoxic concentrations be taken and archived to enable later assessment of true cell density should this be required. The minimum viability per well that has been demonstrated to be scientifically appropriate for data analysis is 80%. Concentrations where cell viability was ≤80% were excluded from data analysis in the OECD validation studies.

If the blank has greater viability than the solvent control, the solvent may be toxic to the cells. If viability is adversely affected at the lowest concentration and continues to decrease with increasing concentration of the test substance, the test substance may be toxic to the cells.

E. Hormone Extraction

If the hormone measurement system is affected by the presence of medium, an extraction procedure is recommended, and the percent recovery of the hormone determined using ^{3}H-labeled hormones. This step is not necessary if it can be demonstrated that the hormone measurement system is not affected by the presence of medium or test chemicals (see Section (h)5 “Hormone Extraction and Analysis” in the Test Guideline).

F. Data Analyses

To evaluate the relative test substance-induced change in hormone production, the hormone concentration results need to be normalized to the mean solvent control value for each assay. EPA recommends that the results be expressed as relative change compared to the solvent control of each exposure plate, and presented as mean ± SD. The Guideline recommends that concentrations that exhibit cytotoxicity approximately >20% be omitted from further evaluation.

If by visual inspection of the well, there appears to be an increase in cell number, this apparent increase needs to be verified. If an increase in cell numbers is verified, EPA recommends that this be stated in the DER and the hormone data be normalized by dividing the hormone concentration by the relative change in the number of viable cells.

IV. STUDY INTERPRETATION

A. Performance Criteria

1. Hormone Measurement Systems

Each laboratory may use a hormone measurement system of its choice for the analysis of the production of testosterone (T) and estradiol (E2) by H295R cells. Prior to the initiation of cell

culture and any subsequent test runs, it is expected that each laboratory demonstrate the conformance of their hormone measurement system (e.g., ELISA, RIA, LC-MS) with the QC criteria defined in Table 2 by analyzing culture medium spiked with an internal hormone control.

Table 2. Performance criteria for hormone measurement systems.

Parameter	Criterion
Method detection limit (MDL)	Testosterone: 100 pg/mL; Estradiol: 10 pg/mL [a]
Spike sample recovery	Supplemented medium are spiked with at least two concentrations of each hormone of interest (T: 500 and 2500 pg/mL; E2: 50 and 250 pg/mL). When analyzed with the hormone measurement assay, the average recovery rates (based on triplicate measures) for the spiked amounts of hormone are expected not to deviate more than 30% from nominal concentrations.
Hormone cross-reactivity (antibody-based systems only)	Cross reactivity is defined within an assay as that which occurs when a hormone other than the one identified as the target hormone is recognized by the antibody. Cross-reactivity is usually included in the instructions with the hormone assay kits. If cross-reactivity of the major hormones of the H-295R cells is not known for the assay system, it must be reported. These include: cholesterol, pregnenolone, progesterone, 11-deoxycorticosterone, corticosterone, aldosterone, 17-alphapregnenolone, 17alpha-progesterone, deoxycortisol, cortisol, DHEA, androstenedione, and estrone.

a Note: Method detection limits shown here are based on the basal hormone production values provided in Table 6, and are performance based. If greater basal hormone production can be achieved, a higher MDL is acceptable provided the criteria in Table 6 are met.

Another important consideration is the identification of the appropriate dilution of the media, in particular the wells containing the solvent control as it is the hormone concentration in these wells that will be used as the basis of comparison for the test chemical. Optimally, the dilution of the solvent control (SC) sample should be such that the hormone value falls near the middle of the linear portion of the standard curve so that there is sufficient room above or below to accurately gage the impact of the test chemical. This will enhance the ability to detect either an increase or decrease in hormone synthesis following exposure to the test chemical.

If antibody-based hormone measurement assays are to be used, prior to initiation of testing, it is recommended that each chemical be tested for potential interference with the hormone measurement system being utilized. It has been previously shown that some chemicals can interfere with antibody-based assays such as ELISAs and RIAs (Shapiro and Page, 1976). It is recommended that the "chemical interference test" be conducted as described in the Test Guideline. Additionally, if the hormone measurement system is affected by the presence of medium, EPA recommends that an extraction procedure be used and the percent recovery of the hormone be determined using ^{3}H-labeled hormones.

2. Laboratory Proficiency Test

As indicated above, before testing unknown substances, a laboratory is typically expected to demonstrate that it is capable of achieving and maintaining appropriate cell culture and test conditions required for the successful conduct of the assay. As the performance of an assay is directly linked to the laboratory personnel conducting the assay, it is recommended that these procedures be repeated if a change in laboratory personnel occurs.

EPA recommends that the proficiency test be conducted under the same conditions used for testing chemicals by exposing cells to six increasing concentrations of the model inducer forskolin (positive control inducer) and the model inhibitor prochloraz (positive control inhibitor). These proficiency tests will ultimately be done under somewhat different exposures than the QC plate used when actually conducting the assay (Tables 3a and 3b) in that the concentrations are more closely distributed about the expected values of the EC50 of forskolin and prochloraz. Separate plates are recommended to be run for forskolin and prochloraz.

Positive Control- Inducer

Table 3a. Dosing schematic (µM) for the placement of the known inducer in a 24-well plate used in the laboratory proficiency study. [a]

	1	2	3	4	5	6
A	DMSO [b]	DMSO [b]	DMSO [b]	Forskolin 10	Forskolin 10	Forskolin 10
B	Forskolin 0.03	Forskolin 0.03	Forskolin 0.03	Forskolin 1.0	Forskolin 1.0	Forskolin 1.0
C	Forskolin 0.3	Forskolin 0.3	Forskolin 0.3	Forskolin 0.1	Forskolin 0.1	Forskolin 0.1
D	Forskolin 3.0	Forskolin 3.0	Forskolin 3.0	Blank	Blank	Blank

a Doses will be administered in DMSO at 0.1% v/v per well. Nothing is added to the blank wells.
b The DMSO solvent control will receive 1 µL of DMSO only.

Positive Control- Inhibitor

Table 3b. Dosing schematic (µM) for the placement of the known inhibitor in a 24-well plate used in the laboratory proficiency study.[a]

	1	2	3	4	5	6
A	DMSO [b]	DMSO [b]	DMSO [b]	Prochloraz 3.0	Prochloraz 3.0	Prochloraz 3.0
B	Prochloraz 0.01	Prochloraz 0.01	Prochloraz 0.01	Prochloraz 0.3	Prochloraz 0.3	Prochloraz 0.3
C	Prochloraz 0.1	Prochloraz 0.1	Prochloraz 0.1	Prochloraz 0.03	Prochloraz 0.03	Prochloraz 0.03
D	Prochloraz 1.0	Prochloraz 1.0	Prochloraz 1.0	Blank	Blank	Blank

a Doses will be administered in DMSO at 0.1% v/v per well. Nothing is added to the blank wells.
b The DMSO solvent control will receive 1 µL of DMSO only.

The hormone effective concentrations, expressed as 50% of the maximum response (EC50s), are calculated using standard procedures (e.g., Probit analysis or moving average method) and compared with the values in Table 4. The EC-50 concentrations are generally expected to fall within the following ranges:

Table 4. EC50 ranges for control substances.

Inducer/Inhibitor	**EC50 (µM)**	
	Testosterone	**Estradiol**
Prochloraz	0.01 – 0.1	0.03 – 0.3
Forskolin	0.2 – 2.0	0.3 – 3.0

3. Assay Quality Control (QC)

The assay QC plate must be included for each assay of a test chemical to determine the accuracy of the assay (i.e., are the expected changes in hormone production identifiable) and for

establishing historical control data. Furthermore, the Guideline recommends the addition of methanol (MeOH) to selected blanks when assessing cell viability. The QC plate (Table 5) is incubated, exposed, and assessed (cell viability/cytotoxicity, hormones extraction, and hormone analysis protocols) in the same manner as test plates. The QC plate will be exposed with a known inducer (forskolin) and inhibitor (prochloraz) of E2 and T synthesis each at two different concentrations of these compounds.

Table 5. Assay QC plate layout.

	1	**2**	**3**	**4**	**5**	**6**
A	Blank [a]	Blank [a]	Blank [a]	Blank [a] + MeOH[b]	Blank [a] + MeOH[b]	Blank [a] + MeOH[b]
B	DMSO 1µL	DMSO 1µL	DMSO 1µL	DMSO + MeOH[b]	DMSO + MeOH[b]	DMSO + MeOH[b]
C	Forskolin 1 µM	Forskolin 1 µM	Forskolin 1 µM	Prochloraz 0.1 µM	Prochloraz 0.1 µM	Prochloraz 0.1 µM
D	Forskolin 10 µM	Forskolin 10 µM	Forskolin 10 µM	Prochloraz 1 µM	Prochloraz 1 µM	Prochloraz 1 µM

a Blank wells receive medium only.

b A 70% methanol (MeOH) solution is added to these wells after termination of exposure and removal of medium. Methanol is used as a positive control for cytotoxicity in the Live/Dead® Cell Viability/Cytotoxicity kit. If an alternative to methanol is used, documentation should be provided to demonstrate that the use of the alternative results in 100% cell death and is suitable for the cytotoxicity test of choice.

The QC plate criteria are presented in Table 6. The minimum basal production (MBP) recommended in the Test Guideline is 500 pg/mL for testosterone and 40 pg/mL for estradiol. If the experimental basal hormone production values are lower than the recommended MPB for testosterone or estradiol, the assay would likely need to meet the other performance specified below to be considered adequately sensitive. For example, if the MBP estradiol level is less than 40 pg/mL, but the basal estradiol production is consistently 2.5 times greater than the minimum detection level (MDL), the E2 values would typically be considered to meet the criteria.

Table 6. QC plate hormone production criteria.

Criterion	Testosterone	Estradiol
Basal production	≥5-fold MDL	≥2.5-fold MDL
Induction (10 µM forskolin)	≥1.5-fold solvent control	≥7.5-fold solvent control
Inhibition (1 µM prochloraz)	≤0.5-fold solvent control	≤0.5-fold solvent control

4. QC Parameters During Testing

In addition to meeting the criteria for the QC plate, other quality criteria that pertain to variation between replicate wells, replicate experiments, linearity and sensitivity of hormone

measurement systems, variability between replicate hormone measures of the same sample and % recovery of hormone spikes after extraction of medium are provided in Table 7.

Table 7. Recommended ranges and/or variation (%) for H295R assay test plate parameters.

Parameter	Comparison	Testosterone	Estradiol
Basal hormone production in solvent controls	Fold-greater than MDL	≥5-fold	≥2.5-fold
Exposure experiments – Within plate CV for solvent controls (replicate wells)	Absolute concentrations	≤30%	≤30%
Exposure experiments – Between plate CV for solvent controls (replicate experiments)	Fold-change	≤30%	≤30%
Hormone measurement system – sensitivity	Detectable fold-decrease relative to solvent controls	≥5-fold	≥2.5-fold
Hormone measurement system – replicate measure CV for solvent controls	Absolute concentrations	≤25%	≤25%
Medium extraction – recovery of internal ^{3}H-standard (if applicable)	CPM	≥65% nominal	

B. Data Interpretation and Graphical Representation

Results should be provided both in graphical (bar graphs representing mean +/-SD) and tabular (lowest concentration showing an effect, direction of effect and strength of maximum response that is part of the dose-response portion of the data) formats. In the graphs, the bars that correspond to statistically significant results should be designated with an asterisk. Examples of graphical representations of the data are provided in the Test Guideline (Section (h) 7 (ii)). Data assessment is typically only considered valid if it has been based on three independently conducted experiments. An experiment or run is considered independent if it has been conducted on a different date and with cells from a different culture plate. Furthermore, the coefficient of variation for the lowest concentration showing a response (or no response if appropriate) among the three experiments is generally expected not to exceed approximately 30%. The concentration range used in Runs 2 and 3 may be tailored on the basis of the results of Run 1 to better define the dose response range containing the lowest concentration that showed an effect.

A chemical is judged to be positive if the fold induction is statistically different from the solvent controls following concentrations that fall within the increasing or decreasing portion of the dose-response curve. Statistically significant increases in fold-induction indicate the chemical is an inducer of one or more enzymes in the steroid synthesis pathway. Statistically significant decreases in fold-induction indicate the chemical is an inhibitor of one or more enzymes in the steroid synthesis pathway. Statistically significant differences at concentrations that do not follow a dose-response curve may be due to random effects; such results are considered to be equivocal. As previously noted, results exceeding the limits of solubility or at cytotoxic concentrations are not to be included in interpreting results.

C. Statistical Analyses

Prior to conducting statistical analyses, the assumptions of normality and homogeneity of variance should be evaluated. Normality can be evaluated using standard probability plots or any other appropriate statistical method (e.g., Shapiro-Wilk's test). If the data are not normally distributed, it is recommended that the data be transformed to approximate a normal distribution. If the data are normally distributed or approximate normal distribution, EPA recommends that differences between chemical treatments and solvent controls be analyzed using parametric tests (e.g., ANOVA followed by Dunnett's test). If the data are not normally distributed, use of an appropriate non-parametric test (e.g., Kruskal Wallis, Steel's Many-one rank test) is recommended. Differences are usually considered significant at $p \leq 0.05$. A summary of the criteria for the evaluation of data is presented in Table 8.

Table 8. Data Categorization Parameters.

Parameter	Criterion
Statistical significance	Response is considered to be statistically significant if the difference from the Solvent Control is $p \leq 0.05$.
Dose response	Data are expected to follow a dose response type profile at non-cytotoxic doses, or doses that do not interfere with the hormone measurement assay (note: response can be biphasic such as an increase at lower and a decrease at higher doses, but changes randomly observed at only a few concentrations within the dose range are to be excluded).
Interference with hormone measurement assay	When marked interference of the chemical of interest with the hormone measurement system utilized occurs (≥ 30% of hormone concentration measured at the same dose at which interference occurred), these data are omitted. In the case of weak to moderate interference (< 30% of hormone concentration measured at the same dose at which interference occurred), results may be corrected for the % interference.
Solubility	The results at concentrations for which cloudiness or a precipitate is observed are not included.
Cell viability	Only non-cytotoxic concentrations (> 80% cell viability) are included.

D. Control Data

A QC plate (previously described in Section III A.3) must be run with every experiment to provide control data for hormone production and cytotoxicity. The QC plate is incubated, exposed and assessed (cell viability/cytotoxicity, hormones extraction, and hormone analysis protocols) in the same manner as test plates. The QC plate contains unexposed wells, wells exposed to solvent only (solvent control), wells exposed to forskolin and prochloraz, in addition to blank and solvent control wells exposed to methanol.

V. STEROIDOGENESIS ASSAY HAZARD CHARACTERIZATION

The Steroidogenesis Assay is intended to identify xenobiotics that affect the steroidogenic pathway. The assay is not intended to identify substances that affect steroidogenesis due to xenobiotic effects on the hypothalamus or pituitary gland. It is intended to be used in

conjunction with other guidelines in the 890 Series to determine on a weight-of-evidence basis if a chemical interacts with components of the endocrine system.

VI. DATA EVALUATION RECORD

Once the study has been reviewed using the principles described in the previous sections of this SEP, a DER will be prepared. A template is available that provides additional guidance for the preparation of the DER.

VII. REFERENCES

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