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

Template version 09/2011

DATA EVALUATION RECORD

STUDY TYPE: Uterotrophic Assay (Rat); OCSPP 890.1600; OECD 440

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

TXR#: *(if applicable)* **CAS#:** [#]

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

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 Unpublished.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]

TEST ORDER #: [Test Order Recipient or the Consortium No.] *(e.g., EDSP-PC Code-###)*

EXECUTIVE SUMMARY: In a Uterotrophic Assay (MRID [number] (if applicable)) conducted to screen for potential **estrogenic activity**, [chemical name (% purity, batch/lot #)] in [vehicle] was administered daily via [(oral gavage or subcutaneous (s.c)] to [#]-day old ovariectomized female [strain] rats at dose levels of 0 (vehicle), [#] or [#] mg/kg/day. To screen for potential **anti-estrogenic activity,** the test substance was also administered daily via [oral gavage or s.c] to [#]- day old ovariectomized female [strain] rats at dose levels of 0 (vehicle), #, [#] or [#] mg/kg/day in conjunction with a daily dose of 17α-ethynyl estradiol (EE) at [#] µg/kg/day by sc injection. For both assays, the animals were dosed for three consecutive days and necropsied approximately 24 hours after the final dose administration to determine wet and blotted uterine weights.

Include a brief summary of the results and a conclusion regarding the estrogenic or anti-estrogenic activity of the test substance. Anti-estrogenic activity is generally indicated by a statistically significant decrease in wet or blotted uterine weight of the treated groups (test substance + EE) compared to the EE only control group. Estrogenic activity is generally indicated by *a significant increase in wet and/or blotted uterine weight compared to the vehicle control. Report any additional data that corroborate or confound the interpretation of the uterine weight data. Discuss any major deficiencies, failure to meet performance criteria or any problems encountered in this study.*

This assay [satisfies/does not satisfy] the Test Order requirement for an Uterotrophic Assay (OCSPP 890.1600). *If it does not satisfy the requirement, concisely list only major deficiencies and refer to deficiency section.*

COMPLIANCE: Signed and dated GLP Compliance and Quality Assurance 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, and relevant physicochemical properties*
Source: *Company*
Lot/Batch #: *include expiration date*
Purity: %
Stability: *How many days and under what condition*
CAS #: *CAS # or Not available*
Structure: *[Structure] or Not available*

2. Reference Estrogen:

17α-ethynyl estradiol (EE)

Supplier *Source/company (City, State [and Country, if outside U.S.A.])*
Lot/Batch #: *include expiration date*
Purity: %
CAS # : 57-63-6

3. Solvent/Vehicle Control:

Supplier Source/company *(City, State [and Country, if outside U.S.A.])*
Lot/Batch #: *include expiration date if applicable*
Rationale (if other than water)
Final concentration

4. Test Animals:

Species: Rat or Mouse *(if mouse, provide rationale)*
Strain: [Sprague-Dawley or Wistar]
Age/weight at dose initiation: [#] days old/ [#] –[#] g females
Source: *Supplier (City, State [and Country, if outside U.S.A.]) The DER should state if animals were born in-house or shipped with dam prior to weaning. If the latter, information on the acclimation period prior to dose initiation should be included. The reviewer should include information on age at receipt, duration of acclimation, and age at initiation and termination of dosing in the appropriate sections of the DER.*

Housing: #/ cage, type of cage, and bedding, etc. [e.g., 3/cage in stainless steel cages, suspended above cage board,]
Housing may be individual for young adult females or in groups of up to three per cage. Group housing is preferred for immature animals. Bedding material should contain a minimal amount of phytoestrogens; and corn cob bedding should generally be avoided, as it affects the estrous cycle in rats via an apparent anti-estrogenic mechanism.

Diet: [Diet name, source, phytoestrogen concentration], *ad libitum*
High levels of phytoestrogens in laboratory diets, primarily from soy and alfalfa products, can increase uterine weights in rodents to an extent high enough to interfere with the Uterotrophic Assay. Furthermore, in immature intact females, high levels of phytoestrogens may accelerate the onset of puberty.

Water: *Source, treatment*,[e.g., Reverse-osmosis filtered tap water], *ad libitum*

Environmental conditions:
Temperature: [#]ºC
Humidity: [#]%
Air changes: [#]/hr
Photoperiod: [#] hrs light/ [#] hrs dark

Acclimation period: (For immature females, note if pups were housed with their dam)

B. METHODS

Any deviations from the suggested guideline methods should be documented.

1. **In Life Dates:** Start: [Month/day/Year] End: [Month/day/year]

2. **Study Design:** *Summarize study design information regarding age of animals at ovariectomy, dosing initiation and termination. Note any concerns or deviations from the guideline that may impact the study. Example text is included below for each model.*

   Following an initial 5-day acclimation period, sexually mature female rats were ovariectomized on post-natal day (PND) [#] *(should be between 42 and 56 days old)* according to standard procedures and allowed 14 days *(7 if mice)* for recovery and regression of uterine weights prior to initiation of dosing. Vaginal smears were taken daily during the last five days of the post-ovariectomy acclimation period and examined microscopically to verify complete removal of the ovaries as indicated by a lack of estrous cycling. The dose administration period was from PND [#] through [#]. Rats were euthanized approximately 24 hours later on PND [#] and necropsied for uterine weight measurements.

   OR

   Following an initial 5-day acclimation period, immature, intact female rats *(or mice)* were administered the test substance from PND [#]-[#]. Rats were euthanized approximately 24 hours after the last dose and necropsied for uterine weight measurements. The pups were housed with their dam prior to weaning.

3. **Animal Assignment:** *Describe procedures for animal assignment (including factors such as randomization, blocking by body weight or day of assignment to allow for staggered necropsy). Note if animals were within acceptable criteria for weight variability at study initiation (i.e., no significant differences among group means and each individual within 20% of the overall mean body weight). If immature animals were used, state if steps were taken to ensure that littermates were not assigned to the same group. Example text is included below.*

   Animals were assigned, stratified by body weight, to the test groups noted in Table 1. Statistical analysis indicated that there were no significant differences between group mean weights at study initiation. Furthermore, the body weight of each animal was within 20% of the overall mean.

Table 1. Study Design[a]

Test Group	Dose (mg/kg/day)	# of Females
Estrogen Agonist Assay		
Vehicle Control	0	6
Low	[#]	6
High	[#]	6
17α-ethynyl estradiol (EE), Reference Estrogen	[#] *(note that EE is used at the µg level)*	6
Anti-Estrogen Assay		
17α-ethynyl estradiol (EE), Reference Estrogen	[#] *(note that EE is used at the µg level)*	6
Low (+EE)	[#]	6
Mid (+EE)	[#]	6
High (+EE)	[#]	6
Vehicle control	0	6

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

Add or delete rows from the table as necessary based on study design, depending on whether assay was testing for estrogen agonistic effects and antagonistic effects. The estrogenic assay uses two dose levels (Low and High) and the anti-estrogenic assay uses three dose levels (Low, Mid and High). The EE group serves as a reference estrogen.

4. **Dose Selection Rationale:** *Briefly describe any range-finding study, including information regarding the study identification (laboratory report or MRID number), study type (i.e., duration, route of administration, number of animals, species), dose levels, effects, and conclusions. The Test Guideline recommends that the highest dose level not exceed the limit dose of 1000 mg/kg/day. See example text below for an estrogenic assay.*

   The dose levels were selected based on the results from a range-finding study (MRID [#]) in which six ovariectomized female rats/dose group received the test substance in corn oil via gavage at doses of 0, 100, 300, or 1000 mg/kg/day for three consecutive days. At 300 and 1000 mg/kg/day, wet and blotted uterine weights were significantly ($p<0.05$) increased over vehicle controls, therefore doses of lower than 300 mg/kg/day were used for the main study.

5. **Dose Preparation and Analysis:** *Information on dose formulations should include: method and frequency of preparation (i.e., for each dose formulation OR via serial dilution, etc.); storage conditions (i.e., duration and temperature); and dose analysis for homogeneity (sampling method); stability (storage temperature and duration), and achieved concentration (dose levels and when analyzed).*

   Dose formulations were prepared [daily OR once prior to initiation of treatment] by initially dissolving the appropriate amounts of test substance in (*type of vehicle/solvent*). The test substance was then mixed with [type of vehicle] for dosing. Doses were adjusted daily based on individual body weight measurements. Homogeneity and stability were tested at *(when, what dose levels, duration, and temperature)*. During the study, samples of dose formulations were analyzed *(when and at what dose levels)* for achieved concentration.

Results of Dose Analysis

Homogeneity: *concentration range as percent of nominal and/or coefficient of variation for different strata (e.g., top, middle, and bottom)*

Stability: *range of values for each temperature and duration tested, expressed as percent of initial (preferable) or percent of nominal concentration*

Concentration: *range of values expressed as percent of nominal*

Example text:
The analytical data indicated that the mixing procedure was adequate and that the variation between nominal and actual dosage to the animals was acceptable. *Describe any deficiencies noted.*

6. **Dosage Administration:** *Summarize dose administration information regarding the test formulations and EE including the route of administration (sc injection preferred, but oral gavage acceptable), dose schedule, and dose volume. The Test Guideline recommends that doses be administered in a volume of ≤5 mL/kg, unless aqueous solutions were used, in which case the Guideline recommends volumes up to 10 mL/kg body weight. The Guideline recommends dosing immature female rats for three consecutive days. A three-day exposure is also recommended for ovariectomized female rats, although a 7-day treatment period may be appropriate as it may improve detection of weakly active substances. If ovariectomized mice are used, a 7-day administration period is strongly recommended to detect any effects. Verify that animals were treated at the same time each day.*

Example text:
Animals were administered the test formulations and/or EE or vehicle daily via s.c. injection for three consecutive days in a dose volume of 5 mL/kg body weight. Dose volumes were adjusted daily based on the concurrent body weight measurement.

7. **Statistics:** *Describe the statistical methods used. Include a statement as to whether or not the Reviewer considers the analyses used to be appropriate; if inappropriate, provide alternative/rationale. If inappropriate refer to the Guideline for the recommended statistical methods and perform the required analysis. Refer to the SEP for details. The criterion for statistical significance should be identified (e.g., $p \leq 0.05$) and groups attaining statistical significance should be denoted. In the DER tables, the asterisk (*) is used as a means of indicating statistical significance. Summary data tables, reporting mean, standard deviation and CV for uterine weights are included for each assay.*

C. METHODS

1. Clinical Examinations: *Describe the frequency and scope of clinical observations (i.e., cage-side check, hand-held physical examination, and/or standard arena assessment). The Test Guideline recommends that clinical observations be conducted at least once daily for mortality and signs of toxicity. Example text is included below.*

Cage-side checks for mortality, moribundity and clinical signs of toxicity were conducted twice daily. Detailed physical examinations were performed prior to initiation of dosing and at termination.

2. Body Weight: *Describe the frequency of body weight measurement. Beginning just prior to initiation of treatment, animals should have been individually weighed each day, both to determine dose volume and effects of treatment on the animals. Example text is included below.*

Animals were weighed at randomization, study initiation, daily throughout the dosing period and at termination.

3. Food Consumption: *Food consumption measurements (on a cage basis), if conducted, should be expressed as g/animal/day. Example text is included below.*

Food consumption was measured for each cage by subtracting the amount of food remaining in the cage from the amount supplied, accounting for spillage. Values were reported as group mean daily food consumption (g/animal/day).

4. Necropsy and Measurement of Uterine Weight: *Briefly, describe the procedures at study termination, including euthanasia, necropsy, measurement of wet and blotted uterus weights, and any optional histopathology. Individual data for uterine weights should have been reported to the nearest 0.1 mg. Unless there are deviations from the standard operating procedures, it is generally unnecessary to provide more than a brief summary of the methods for dissection and weighing of the uterus. Note any deviations from the Test Guideline, especially any procedures that may add to variability in uterus weight. (e.g., loss of fluids, desiccation, etc.). Example text is included below.*

On PND [#] (approximately 24 hours after final administration of the test substance), all surviving animals were euthanized (*describe method)*, exsanguinated, and subjected to a gross necropsy. Dissection of the uterus was performed according to the U.S. EPA Guideline. Briefly, the vagina was removed just below the cervix in order to retain the luminal fluid in the uterus. The "wet" uterus (i.e., containing the luminal fluid) was weighed. Subsequently, the uterine horns were cut longitudinally and gently blotted with moist filter paper to remove the luminal fluid while preventing desiccation and the blotted uterus was weighed.

5. Microscopic Examination: *(If performed). Following fixation in10% buffered formalin and HE-staining, the vagina may be examined histologically for keratinization and cornification (Jones and Edgren, 1973). In addition, morphometric measurement of endometrial epithelium may be done for quantitative comparison*.

II. RESULTS

A. OBSERVATIONS

1. Mortality: *Report any mortality and assess whether any animal deaths were due to treatment with the test substance. If treatment-related mortality occurred, the assay should have been repeated at lower doses because the maximum tolerated dose was exceeded. However, mortality may occur that is unrelated to treatment (e.g., due to gavage error or occurring at the low dose but not at the high dose). Nevertheless, the impact that these deaths have on the assay's interpretation and acceptability should be carefully scrutinized because smaller sample sizes resulting from mortality reduces the power to discern statistically significant differences. Example text is included below.*

All animals survived until scheduled termination.

OR

One animal at the low-dose group (Animal No. [#]) was found dead on Day 2 of dosing. Gross examination of this animal at necropsy revealed a punctured lung, implicating gavage error. All other rats survived until scheduled sacrifice.

2. Clinical Signs of Toxicity: *Report any clinical signs of toxicity, including information on the nature, incidence, severity, onset, and duration. As with the mortality data, the reviewer should assess whether the findings are considered adverse and related to treatment and if they had an impact on dose selection*

Describe results.

Table 2. Incidence of Clinical Observations in the Estrogen Agonist Assay [a]

Observation	Dose (mg/kg/day)							
	Vehicle Control		Low (#)		High (#)		Reference Estrogen EE (#)	
	# Observed	# Examined	# Observed	# Examined	# Observed	# Examined	# Observed	# Examined

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

Table 3. Incidence of Clinical Observations in the Anti-Estrogenic Assay [a]

Observation	Dose (mg/kg/day)									
	Vehicle Control		EE		Low (#) (+EE)		Mid (#) (+ EE)		High (#) (+EE)	
	# Observed	# Examined	# Observed	# Examined	# Observed	# Examined	# Observed	# Examined	# Observed	# Examined

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

If clinical signs of toxicity are not observed include the following example text.

No clinical signs of toxicity were observed in animals for any dose groups.

B. <u>BODY WEIGHT AND WEIGHT GAIN</u>: *Some form of the following tables is mandatory. At a minimum, body weights should be reported for the first and final days of dosing, along with the body weight gain for the overall study (Days 1-3 or 1-7). Additionally, data from any days on which statistically significant decreases in body weights were observed should be included in the table to highlight treatment-related effects. Any effects of treatment on body weights should be described in text, including information on statistical significance, magnitude difference from controls, and the onset and duration of the decreases. Treatment-related, statistically significant decreases in body weights of >10% compared to controls can indicate excessive toxicity and may affect the acceptability of the assay. Example text is included below.*

Selected body weight and body weight gain data are presented in Tables 4 and 5. Body weights were decreased ($p<0.01$) by [#]% on Day 3 in the high dose group compared to controls, resulting in a decrease ($p<0.05$) of [#]% in overall body weight gain. Body weights in the low- and mid-dose groups were comparable to controls throughout the duration of the assay.

Table 4. Selected Group Body Weights and Cumulative Body Weight Gains (g) In the Estrogen Agonist Assay [a]

Study Day #	Dose (mg/kg/day)										
	Vehicle Control			Low (#)			High (#)			Reference Estrogen EE (#)	
	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean
#											
#											
#											
Body Weight Gain (# - #)											

a Data were obtained from page [#] of the study report.
N= No. of animals in the group
SD = Standard Deviation
* Significantly different from controls at $p<0.05$
**Significantly different from controls at $p<0.01$

Table 5. Selected Group Body Weights and Cumulative Body Weight Gains In the Anti-Estrogen Assay (g) [a]

Study Day	Dose (mg/kg/day)														
	Vehicle Control			EE (#)			Low (#) (+EE)			Mid (#) (+ EE)			High (#) (+EE)		
	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD
#															
#															
#															
Body Weight Gain (# - #															

a Data were obtained from page [#] of the study report.
N= No. of animals in the group
SD = Standard Deviation
* Significantly different from controls at p<0.05
**Significantly different from controls at p<0.01

C. FOOD CONSUMPTION

If measured, include enough food consumption information to document any effects observed, or as necessary to explain effects on body weight.

Table 6. Food Consumption (g/kg/day) in the Estrogen Agonist Assay[a]

Study Day #	Dose (mg/kg/day)										
	Vehicle Control			Low (#)			High (#)			Reference Estrogen EE (#)	
	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean
#											
#											
#											
Overall											

a Data were obtained from page [#] of the study report.
N= No. of animals in the group
SD = Standard Deviation
* Significantly different from controls at p<0.05
**Significantly different from controls at p<0.01

Table 7. Food Consumption (g/kg/day) in the Anti-Estrogen Assay[a]

Study Day	Dose (mg/kg/day)														
	Vehicle Control			EE (#)			Low (#) (+EE)			Mid (#) (+ EE)			High (#) (+EE)		
	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD
#															
#															
#															
Body Weight Gain (#-#															

a Data were obtained from page [#] of the study report.
N= No. of animals in the group
SD = Standard Deviation
* Significantly different from controls at p<0.05
**Significantly different from controls at p<0.01

D. PATHOLOGY

1. Uterine Weights: *Some form of the following table(s) is (are) mandatory*

Table 8. Uterine Weights from Estrogen Agonist Assay in [SD or Wistar] Rats[a]

Parameter	Dose (mg/kg/day)									
	Vehicle Control			Low (#)		High (#)		Reference Estrogen EE (#)		
	N	Mean	SD	Mean	SD	Mean	SD	N	Mean	SD
Terminal BW										
Wet, absolute (mg)										
Wet, relative (%)										
Blotted, absolute (mg)										
Blotted, relative (%)										

a Data were obtained from page [#] of the study report.
BW= body weight
N= No. of animals in the group
SD = Standard Deviation
* Significantly different from controls at p<0.05
**Significantly different from controls at p<0.01

Table 9. Uterine Weights from Anti-Estrogenic Assay in [SD or Wistar] Rats[a]

Parameters	Dose (mg/kg/day)														
	Vehicle Control			EE (#)			Low (#) (+EE)			Mid (#) (+ EE)			High (#) (+EE)		
	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD
Terminal BW															
Wet, absolute (mg)															
Wet, relative (%)															
Blotted, absolute (mg)															
Blotted, relative (%)															

a Data were obtained from page [#] of the study report.
BW= body weight
N= No. of animals in the group
SD = Standard Deviation
* Significantly different from controls at p<0.05
**Significantly different from controls at p<0.01

2. **Microscopic Examination:** *Report histopathology data from the uterus, ovaries and/or cervix which corroborate the increased uterine weight as estrogenic responses*.

Table 10. Microscopic Examination of the Vagina in the Estrogen Agonist Assay[a]

Parameter	Dose (mg/kg/day)							
	Vehicle Control		Low (#)		High (#)		Reference Estrogen EE (#)	
	# Observed	# Examined	# Observed	# Examined	# Observed	# Examined	# Observed	# Examined
Keratinization								
Cornification								

a Data were obtained from page [#] of the study report.
* Significantly different from controls at p<0.05
**Significantly different from controls at p<0.01

Table 11. Microscopic Examination of the Vagina in the Anti -Estrogen Assay[a]

Observation	Dose (mg/kg/day)									
	Vehicle Control		EE		Low (#) (+EE)		Mid (#) (+ EE)		High (#) (+EE)	
	# Observed	# Examined	# Observed	# Examined	# Observed	# Examined	# Observed	# Examined	# Observed	# Examined
Keratinization										
Cornification										

a Data were obtained from page [#] of the study report.
* Significantly different from controls at p<0.05
**Significantly different from controls at p<0.01

Table 12. Microscopic Examination of the Ovaries in the Estrogen Agonist Assay[a]

Parameter	Dose (mg/kg/day)							
	Vehicle Control		Low (#)		High (#)		Reference Estrogen EE (#)	
	# Observed	# Examined	# Observed	# Examined	# Observed	# Examined	# Observed	# Examined
Epithelial cell height								

a Data were obtained from page [#] of the study report.
* Significantly different from controls at p<0.05
**Significantly different from controls at p<0.01

Table 13. Microscopic Examination of the Ovaries in the Anti -Estrogen Assay[a]

Observation	Dose (mg/kg/day)									
	Vehicle Control		EE		Low (#) (+EE)		Mid (#) (+ EE)		High (#) (+EE)	
	# Observed	# Examined	# Observed	# Examined	# Observed	# Examined	# Observed	# Examined	# Observed	# Examined
Epithelial cell height										

a Data were obtained from page [#] of the study report.
* Significantly different from controls at $p<0.05$
**Significantly different from controls at $p<0.01$

III. DISCUSSION AND CONCLUSIONS

A. <u>INVESTIGATOR'S CONCLUSIONS</u>: *Concisely summarize the investigators' conclusions regarding the estrogenic or anti-estrogenic effects of the test compound on uterine weights and any optional measurements.*

B. <u>AGENCY COMMENTS</u>: *Summarize the results from the Uterotrophic Assay, emphasizing estrogenic or anti-estrogenic effects on the wet and blotted uterine weights. Discuss the adequacy of dose selection. Discuss any relevant findings in optional parameters such as corroborating histopathology in the uterus, cervix, and/or vagina. If any systemic toxicity was observed (e.g., mortality, clinical signs of toxicity, or decreased body weights or food consumption), integrate these effects into the interpretation of the assay (i.e, determine if the dosing was so high that it precludes meaningful interpretation of the uterus weight data). Note any discrepancies with investigators' conclusions (e.g. rationale for acceptability or necessity for repeating assay).*

C. <u>STUDY DEFICIENCIES</u>: *List each deficiency (distinguishing between major and minor ones) and indicate what data are required to resolve the deficiency.*