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: Female Pubertal Assay; OCSPP 890.1450; OECD None.

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))* (% 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 Unpublished.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 Female Pubertal Assay (MRID [number] (if applicable)), [#] [strain] rats/dose group were treated daily via [oral gavage] with [chemical name (% purity, batch/lot #)] in [vehicle] at doses of [0] ([vehicle]), [#] or [#] mg/kg/day from post-natal day (PND) [22] to [42]. Animals were examined for vaginal opening daily beginning on PND 22, and weight at day of attainment was recorded. Following sacrifice on PND [42] or [43], total thyroxine (T4) and thyroid stimulating hormone (TSH) levels were analyzed using a [type] assay. Urogenital organ weights were recorded, and microscopic examination of the [ovaries, uterus, thyroid, liver, pituitary, adrenal and kidneys] were performed.

Provide a brief summary of the results and a concise discussion. 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 a Female Pubertal Assay (OCSPP 890.1450). *If it does not satisfy the requirement, concisely list only major deficiencies or refer to deficiency section.*

COMPLIANCE: Signed and dated GLP and Quality Assurance statements [were /were not] provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Facility: *Name of the Facility*

- **Location:** *Location of the Facility*
- **Study Director:** *Name*
- **Other Personnel:** *Name and study responsibility*
- **Study Period:** *Study start and end dates*

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

- **Description:** *e.g. technical, nature, color, molecular weight, and relevant physiochemical properties*
- **Source:** *Company (and catalog number if available*
- **Lot/Batch #:** *include expiration date*
- **Purity:** %
- **Stability:** *Provide available data*
- **CAS #:** *CAS # or* Not available
- **Structure:** *[Structure] or* Not available

3. Vehicle: [Corn oil, Water, or Carboxymethylcellulose]. *Solvents such as Acetone and DMSO should be avoided. Include expiration date (if applicable)*

4. Test Animals:

- **Species:** Rat
- **Strain:** Sprague-Dawley (preferred), Wistar, or Long-Evans
- **Age/Weight at Study Initiation:** PND [42]/[#] –[#] g females only
- **Source:** *Supplier (city, state [and country, if outside U.S.)*
- **Housing:** *##/cage, type of cage, and bedding, etc.* [e.g., 3/cage in stainless steel cages, suspended above cage board,]
  Recommended housing is 2-3 animals of the same dose group/cage with heat-treated laboratory-grade wood shavings (not cedar) as bedding. Corn cob bedding is NOT recommended due to potential to disrupt endocrine activity.
- **Diet:** *Diet name, source, ad libitum* Phytoestrogen content [#] µg of genistein equivalents/gram diet
- **Water:** *Source, treatment,*[e.g., Reverse-osmosis filtered tap water], *ad libitum*
- **Environmental Conditions:**
  - **Temperature:** [#]°C
  - **Humidity:** [#]%
  - **Air changes:** [#]/hr
  - **Photoperiod:** [#] hrs light/ [#] hrs dark

B. STUDY DESIGN

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

2. Mating: *Describe procedure for obtaining juvenile animals (e.g., bred in-house, including determination of confirmation of pregnancy, OR received pregnant time-mated dams from supplier on GD 7, 8, 9, or 10) and standardizing litter size to 8-10 pups by culling on PND 3-5. Example text is included below.*

Sexually mature males and nulliparous female rats of the same strain were mated (1:1) in-house, and mating was confirmed by presence of a copulatory plug and/or examination of sperm in a daily vaginal smear. The day on which positive evidence of mating was observed was designated as gestation day (GD) 0. Litters with more than 8 pups were standardized by culling on PND 4 to 8 pups per litter.

OR

Time-mated pregnant dams were received from the supplier on GD [7, 8, 9, or 10 (must be on same day for each study)]. Litters with more than 8 pups were standardized by culling on PND 4 to 8 pups per litter. *Cross fostering is unacceptable*.

3. **Animal Assignment:** Animals were assigned *(note how assigned, e.g., randomized complete block design (time-separated necropsy is the blocking factor))* to the test groups noted in Table 1. Littermates [were/were] not assigned to the same treatment group. *The treatment groups include: (1) the vehicle-treated and (2) xenobiotic-treated with at least two dose levels.*

TABLE 1. Study Design [a]

Test group	Dose (mg/kg/day)	# of Females
Control	0	15
Low	[#]	15
High	[#]	15

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

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, species), dose levels, effects, and conclusions. The guideline recommends that the highest dose level be at or just below the Maximum Tolerated Dose (MTD) but need not exceed the Limit Dose (1000 mg/kg/day); however, typically, the Agency also considers the toxicity profile of the chemical (i.e., cholinesterase inhibition, target organ toxicity, etc.) in dose selection. The second dose level should typically be spaced to produce a lesser degree of toxicity relative to the high dose unless justification is provided for testing at a different level. Example text is provided below.*

The dose levels were selected based on the results from a [*appropriate study type*] toxicity study. In a Developmental Neurotoxicity Study (MRID No. [#]), maternal animals were administered the test substance by gavage in corn oil at doses of 0, 100, 300, or 1000 mg/kg/day from gestation day (GD) 6 to lactation day (LD) 11. On PND 21, there was a decrease in pup body weight at the highest dose tested.

5. **Dose Preparation and Analysis:** Dose formulations were prepared daily by mixing appropriate amounts of test substance with [vehicle]. Homogeneity and stability were tested at *(state when, what dose levels, duration, and temperature)*. During the study, samples of dose formulations were analyzed *(state 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*

The analytical data indicated that the mixing procedure was [adequate/inadequate] and that the variation between nominal and actual dosage to the animals was [acceptable/unacceptable]. *Describe any deficiencies noted.*

6. **Dosage Administration:** All doses were administered once daily by gavage, from PND 22 through PND 42, in a volume of [#] *(recommended range from 2.5 to 5.0)* mL/kg of body weight. Dosing was performed between [0700] and [0900] hours daily.

7. **Statistics:** *The following describes the statistical analyses recommended by the test guideline and may be used by the reviewer for verification. The DER should list parameters that were analyzed and the statistical methods used and include a statement as to whether or not the Reviewer considers the analyses used to be appropriate. If inappropriate, provide alternative/rationale. The data should be analyzed for normal distribution and homogeneity of variance in order to satisfy the assumptions of Analysis of Variance (ANOVA) and Analysis of Covariance (ANCOVA). It is recommended that all data except histology and cyclicity evaluations (i.e., initial body weight [PND 22], body weight, body weight gain, age and body weight at vaginal opening, terminal body weight, organ weights, and serum hormones) should be analyzed by ANOVA. If the study was conducted in blocks, then it is recommended that the analysis be conducted using a two-way ANOVA with Block and Treatment as main effects. It is recommended that age and body weight at vaginal opening and all organ weights also be analyzed by ANCOVA, using the body weight at PND 22 as the covariate. When statistically significant effects among groups are observed ($p<0.05$), appropriate pair-wise comparison tests would be needed in order to identify which dose groups are significantly different from the control group. Where there is heterogeneity of variance, data should be transformed appropriately to achieve homogeneous variances prior to ANOVA/ANCOVA, or analyzed using an appropriate nonparametric test. However, non-parametric analysis would generally be the recommended method of last resort as it does not allow analysis of covariation. In addition to ANOVA and ANCOVA, it is recommended that the unadjusted and adjusted values should be examined for linear trend with dose level. It is recommended that cycling status (cycling vs. not cycling) and percent of animals cycling regularly be analyzed using Chi-square analysis.*

C. METHODS

1. **Mortality and Clinical Examinations:** All animals were examined *[frequency; e.g. twice daily]* for mortality and moribundity. Clinical examinations were conducted [*frequency*].

2. **Body Weight:** Animals were weighed daily prior to dosing.

3. **Vaginal Opening:** Beginning on PND [22], all animals were examined daily for onset of vaginal opening. *(The Test Guidelines recommend that the appearance of a small "pin hole", a vaginal thread, and complete vaginal opening be recorded for all days they are observed.)* Age and weight on the day of completion of vaginal opening were recorded.

4. **Estrous Cyclicity:** Beginning on the day of vaginal opening, up to and including the day of necropsy, daily vaginal smears were obtained and evaluated for the presence of leukocytes, nucleated epithelial cells, or cornified epithelial cells. The vaginal smears were classified as diestrus (predominance of leukocytes mixed with some cornified epithelial cells), proestrus (predominance of clumps of round, nucleated epithelial cells), or estrus (predominance of cornified epithelial cells). The age at first vaginal estrus was recorded. *A typical cycle consists of two or three days of diestrus, one day of proestrus, and one or two days of estrus. The study should normally include detailed information on estrous cyclicity for all animals and summary information for the dose groups, including (i) the mean age at first vaginal estrus, (ii) the mean cycle length for each group, (iii) the percent of each group cycling, (iv) the percent of each group cycling regularly, and (v) the stage of the cycle at the time of necropsy.*

5. **Sacrifice and Pathology:** On the day of termination, rats were removed one at a time from their home cages to a separate room for euthanasia and terminal procedures so as not to induce stress-related responses in other animals which may affect hormone measurements. All surviving animals were sacrificed *[(describe method; preferred method of sacrifice is by injectable anesthetic, followed by immediate decapitation; carbon dioxide is not an acceptable anesthetic)]* on PND [42] approximately 2 hours post-dosing. *(The guideline recommends sacrificesbe completed by 1300 hours due to normal diurnal fluctuation in thyroid hormone levels)* Blood from the trunk of the animals was collected immediately into serum separation tubes, processed [method] and stored at [temperature]*(should be -20°C or colder)* for subsequent hormone and clinical chemistry evaluations. (*Historical control data should be provided.)*

   a. Hormone Analysis: Total thyroxine (T4) and thyroid stimulating hormone (TSH) levels were analyzed using *[assay type; radioimmunoassay (RIA), immunoradiometric assay (IRMA), enzyme-linked immunosorbent assay (ELISA), or time-resolved immunofluorescent procedures.]*

b. Clinical Chemistry: The following CHECKED (X) parameters were examined.

X	ELECTROLYTES	X	OTHER
	Calcium		Albumin
	Chloride		Creatinine*
	Magnesium		Urea nitrogen*
	Phosphorus		Total cholesterol
	Potassium		Globulins
	Sodium		Glucose
	ENZYMES		Total bilirubin
	Alkaline phosphatase (ALK)		Total protein
	Cholinesterase (ChE)		Triglycerides
	Creatine phosphokinase		Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)		
	Alanine aminotransferase (ALT/also SGPT)		
	Aspartate aminotransferase (AST/also SGOT)		
	Sorbitol dehydrogenase		
	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

* Recommended for the pubertal assay in female rats based on guideline 890.1450.

c. Organ Weights and Histopathology: The following CHECKED (X) tissues were collected and weighed. The (XX) organs, in addition, were subjected to histological examination *(note if not all collected tissues were examined)*.

X	UROGENITAL	X	OTHER
	Ovaries (paired, without oviducts)*+		Thyroid*+
	Uterus*		Liver*
	Kidneys (paired)*+		Adrenals (paired)*
			Pituitary*

* Weights required based on guideline 890.1450
\+ Histopathological examination required based on guideline 890.1450

All organs collected, except the thyroid/trachea and uterus were weighed prior to fixation. Paired organs (kidneys, adrenals, and ovaries) were weighed together. The uterus and cervix were separated from the vagina and weighed. The uterus was weighed again following removal of the fluid in the lumen (blotted weight).

The ovaries [left or right] *(should be from the same side of each animal; report any deviations)* and uterus were fixed in 10% buffered formalin and stored in 70% ethanol prior to embedding. The thyroid (with parathyroid) and kidneys were fixed in 10% buffered formalin for at least 24 hrs. Following fixation, the thyroid was dissected from the trachea. All collected tissues were routinely processed into paraffin blocks, sectioned, stained with hematoxylin and eosin, and examined microscopically.

This is the information that you would be found in the study report conducted following guideline recommendations. Thyroid sections are subjectively evaluated for follicular cell height and colloid area, preferably using a five point grading scale (1 = shortest/smallest; 5 = tallest/largest), and any abnormalities/lesions noted. At least two sections from each of the two lobes of the thyroid should be examined in order to obtain representative sample of the thyroid tissue from each lobe. Note whether ovarian histology included evaluations of

any abnormalities/lesions (such as ovarian atrophy) and follicular development, including: presence/absence of tertiary/antral follicles, presence/absence of corpora lutea, changes in corpus luteum development, changes in number of both primary and atretic follicles. Five random sections should be evaluated using the method of Smith et al. (1991). Uterine histology should document cases of uterine hyper- or hypotrophy as characterized by changes in uterine horn diameter and myometrial, stromal, or endometrial gland development. The histological assessment should take into account the stage of the estrous cycle of the female at the time of necropsy, as ovarian and uterine cellular changes are dependent upon endocrine status. Report the histopathological findings in Section II.G.

II. RESULTS *Describe findings include tables if needed; tables are recommended to depict any treatment-related findings, thus limiting use of text to highlight specific points.*

A. Mortality: *Report any mortality and assess whether any animal deaths were due to treatment with the test substance or other circumstances (e.g., gavage errors). Example text is included below.*

All animals survived until scheduled termination.

OR

One animal in the low-dose group (Animal No. [#]) was found dead on PND 25. Gross examination of this animal at necropsy revealed a punctured lung, implicating gavage error. All other rats survived until scheduled sacrifice.

B. 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] [include table only if treatment-related effects were seen]

TABLE 2. Incidence of Clinical Observations [a]

Observation	Dose Level (mg/kg bw/day)					
	Vehicle Control		Low [#]		High [#]	
	# Observed	# Examined	# Observed	# Examined	# Observed	# Examined
[observation type]						

a Data were extracted 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.

C. General Growth and Vaginal Opening: Body weights, body weight gains, age of attainment of vaginal opening and weight at day of attainment are presented in Table 3 below. *This table is **MANDATORY**.*

TABLE 3. General Growth and Vaginal Opening (VO)[a].

Parameter Evaluated		Vehicle Control				Low Dose ([#] mg/kg/day)				High Dose ([#] mg/kg/day)			
		Number of Animals Examined	Mean	SD	CV	Number of Animals Examined	Mean	SD	CV	Number of Animals Examined	Mean	SD	CV
Initial body weight (PND 22; g)	U												
	A												
Body weight at vaginal opening (g)	U												
	A												
Final body weight (g)	U												
	A												
Final body weight (% of control)	U												
	A												
Body weight gain (final – initial; g)	U												
	A												
Age at vaginal opening (PND)	U												
	A												
Proportion unopened (#/N)													

a Data were obtained from page [#] of the study report, and are the average of [#] female rats per dose group.
U = Unadjusted for body weight on PND 22
A = Adjusted for body weight on PND 22
SD = Standard Deviation
CV = Coefficient of Variation
* Significantly different from controls at $p<0.05$.
NA Not applicable

D. Organ Weights: Organ weights at necropsy are presented in Table 4 below. *(This table is **MANDATORY**. Describe any treatment-related effects.)*

TABLE 4. Organ Weights at Necropsy[a].

Organ		Vehicle Control				Low Dose ([#] g/kg/day)				High Dose ([#] mg/kg/day)			
		Number of Animals Examined	Mean	SD	CV	Number of Animals Examined	Mean	SD	CV	Number of Animals Examined	Mean	SD	CV
Liver (g)	U												
	A												
	R												
Kidneys (g)	U												
	A												
	R												
Pituitary (mg)	U												
	A												
	R												
Adrenals (mg)	U												
	A												
	R												
Ovaries (mg)	U												
	A												
Uterus, wet (mg)	U												
	A												
Uterus, blotted (mg)	U												
	A												
Thyroid, fixed (mg)	U												
	A												

a Data were obtained from page [#] of the study report, and are the average of [#] female rats per dose group.
U = Unadjusted for body weight on PND 22
A = Adjusted for body weight on PND 22
SD = Standard Deviation
CV = Coefficient of Variation
R = Organ-to-body weight ratio (relative to body weight)
* Significantly different from controls at $p<0.05$.

E. Estrous Cyclicity: Estrous cycle data are provided in Table 5 below. *Include some form of this table or some other method of providing this information in this DER. Describe any treatment-related effects*

TABLE 5. Estrous Cyclicity[a]

Dose Level (mg/kg/day)	Number of Animals (N)	Mean Age at First Vaginal Estrus (PND)	Mean Cycle Length (days)	Cycling (%)	Regularly Cycling (%)	Cycle Status at Necropsy (# Females)			
						Diestrus	Proestrus	Estrus	Not Cycling
Vehicle									
Low Dose									
High Dose									

a Data were obtained from page [#] of the study report, and are the average of [#] female rats per dose group.
* Significantly different from controls at $p<0.05$.

F. Clinical Chemistry and Hormone Levels: Mean hormone levels are presented in Table 6 below. *[Optional text: In addition, the study report provided normal ranges for the clinical chemistry and or hormone measures. Appropriate data are appended to this DER.]* *(This table is **MANDATORY**. Add rows as necessary to include any additional parameters examined. Describe any treatment-related effects)*

TABLE 6. Hormone Levels and Clinical Chemistry [a]

Parameter Evaluated	Vehicle Control				Low Dose ([#] mg/kg/day)				High Dose ([#] mg/kg/day)			
	Number of Animals Examined	Mean	SD	CV	Number of Animals Examined	Mean	SD	CV	Number of Animals Examined	Mean	SD	CV
Hormones												
Serum T_4, Total (µg/dL)												
Serum TSH (ng/mL)												
Clinical Chemistry												
Creatinine (µmol/L)												
Blood urea nitrogen (mmol/L)												

a Data were obtained from page [#] of the study report, and are the average of [#] female rats per dose group.
SD = Standard Deviation
CV = Coefficient of Variation
* Significantly different from controls at $p<0.05$.

G. Histopathology: The incidence of histopathological findings of the thyroid gland are presented below in Table 7. (*This table is MANDATORY; limit text to integration of findings, highlights; relate with other findings, as appropriate).*

TABLE 7. Incidence of Histopathological Lesions of the Thyroid Gland [a]

Treatment Groups	Parameter Evaluated											
	Colloid Quality			Follicular Cell Height (Increase)			Follicular Cell Height (Decrease)			Follicular Cell Shape		
	Severity[b]	Incidence		Severity	Incidence		Severity	Incidence		Severity	Incidence	
		O	E		O	E		O	E		O	E
Vehicle Control	0			0			0			0		
	1			1			1			1		
	2			2			2			2		
	3			3			3			3		
Low Dose ([#] mg/kg/day)	0			0			0			0		
	1			1			1			1		
	2			2			2			2		
	3			3			3			3		
High Dose ([#] mg/kg/day)	0			0			0			0		
	1			1			1			1		
	2			2			2			2		
	3			3			3			3		

a Data were obtained from page [#] of the study report.
b Thyroid histopathology is graded 0 – 3 based on severity: 0=Not remarkable, 1=Mild, 2=Moderate, 3=Severe. See OECD No. 82 for reference.
O = No. Observed
E= No. Examined

The incidence of histopathological findings of the ovaries, uterus and kidneys are presented in Table 8.

TABLE 8. Incidence of Histopathological Lesions of the Ovaries, Uterus and Kidney[a]

Findings	Dose Level (# mg/kg bw/day)					
	Vehicle Control		Low (#)		High (#)	
	# Observed	# Examined	# Observed	# Examined	# Observed	# Examined
Ovaries						
[observation type]						
Uterus						
[observation type]						
Kidney						
[observation type]						

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

III. DISCUSSION AND CONCLUSIONS

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

B. AGENCY COMMENTS: *Briefly summarize the results and discuss the following:*

- *Evaluate the adequacy of the dose levels tested.*
- *Discuss the systemic toxicity observed (i.e., survival, body weight, body weight gain, target organ toxicity etc)*
- *Was age and weight at vaginal opening within acceptable ranges? Report findings relative to performance criteria?*
- *Were there any effects on estrous cyclicity?*
- *Were there any changes in organ weights compared to controls, Report findings relative to performance criteria?*
- *Were there any effects on hormone levels?*
- *Were there any effects on clinical chemistry parameters?*
- *Were there any changes in histopathological findings?*

C. STUDY DEFICIENCIES: *List each deviation from the protocol and classify the deviation as major or minor. Also report any rationale provided by the investigator's for the deviation. Similarly list, classify, and discuss all other deficiencies with the conduct, results, and reporting of the study. Discuss the possibility of resolving the deficiencies and what would be required. Major deficiencies may be presented and discussed in paragraph form, whereas minor deficiencies can be presented in a bulleted list.*