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: Male Pubertal Assay; OCSPP 890.1500

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 Unpublished.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 Male 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) [23] to [53]. Animals were examined for preputial separation (PPS) daily beginning on PND 30 and weight at day of attainment was recorded. Following sacrifice on PND [53] or [54], total serum testosterone, thyroxine (T4), and thyroid stimulating hormone (TSH) levels were analyzed using a [type] assay. Urogenital organ weights were recorded and microscopic examination of the [testes, epididymides, prostate, thyroid, 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 Male Pubertal Assay in rats (OCSPP 890.1500). *If it does not satisfy the requirement, concisely list only the major deficiencies and 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, odor, and molecular weight*
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 [#] /[#] –[#] g males 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.

3. **Animal Assignment:** Offspring were weaned on PND [21] and weighed. The rats from the extreme ends of the weight range were eliminated to achieve a mean body weight and variance across dose groups at study initiation. 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 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 Males
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 range-finding study (MRID [#]) in which [15] rats/sex/dose group were administered the test substance in corn oil via gavage at doses of 0, 500, or 1000 mg/kg/day from PND [23-53]. At 1000 mg/kg/day, body weights were significantly decreased by 15-18% ($p<0.05$) compared to controls beginning on PND 42, resulting in a decrease of 16% $p<0.05$) in terminal body weight. Body weights at 500 mg/kg/day were comparable to controls throughout the study. There were no mortalities, clinical signs of toxicity, or effects on BUN or creatinine at either dose.

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 23 through PND 53, 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 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 (i.e., initial body weight [PND 23], body weight gain, age and body weight at preputial separation, terminal body weight, organ weights, and serum hormones be analyzed by ANOVA. If the study was conducted in blocks, then the recommended analysis is to use to a two-way ANOVA with Block and Treatment as main effects. It is recommended age and body weight at preputial separation and all organ weights also be analyzed by ANCOVA, using the body weight at PND 23 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 group is 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 method of last resort as it does not allow analysis of covariance. In addition to ANOVA and ANCOVA, it is recommended that the unadjusted and adjusted values be examined for linear trend with dose level.*

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. **Preputial Separation (PPS):** Beginning on PND [30], all animals were examined daily for onset of PPS. Age and weight at on the day of completion of PPS were recorded.

4. **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 is decapitation without anesthesia)*] on PND [53] approximately 2 hours post-dosing. *(The guideline recommends sacrifices be 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 testosterone, total thyroxine (T_4), 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
	Calcium
	Chloride
	Magnesium
	Phosphorus
	Potassium
	Sodium
	ENZYMES
	Alkaline phosphatase (ALK)
	Cholinesterase (ChE)
	Creatine phosphokinase
	Lactic acid dehydrogenase (LDH)
	Alanine aminotransferase (ALT/also SGPT)
	Aspartate aminotransferase (AST/also SGOT)
	Sorbitol dehydrogenase
	Gamma glutamyl transferase (GGT)
	Glutamate dehydrogenase

X	OTHER
	Albumin
	Creatinine*
	Urea nitrogen*
	Total cholesterol
	Globulins
	Glucose
	Total bilirubin
	Total protein
	Triglycerides
	Serum protein electrophoresis

* Recommended based on guideline 890.1500.

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
	Testes (left and right separately)*+		Thyroid*+
	Epididymides (left and right separately)*+		Liver*
	Seminal vesicle plus coagulating glands (with and without fluid)*		Adrenals (paired)*
	Ventral prostate*		Pituitary*
	Dorsolateral prostate*		
	Levator ani/bulbocavernosus (LABC) muscle complex*		
	Kidneys (paired)*+		

* Weights required based on guideline 890.1500
\+ Histopathological examination required based on guideline 890.1500

The testis and epididymis [left or right] (*the guideline recommends that both be from the same side of each animal in the study; report any deviations)* and kidneys were weighed prior to fixation. Following weighing, the testis and epididymis were fixed in Bouin's solution overnight *(not to exceed 24 hrs)*. The thyroid (with parathyroid) was collected with the trachea and fixed in 10% buffered formalin for at least 24 hrs. Following fixation, the thyroid was dissected free of the trachea and weighed. 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. Guidance related to the histological evaluation of the testis and epididymis is given in EPA's Health Effects Test Guideline OPPTS 870.3800: Reproduction and Fertility Effects (US EPA, 1998). Report the histopathological findings in Section II.F.

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).*

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.*

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 group.

C. General Growth and Preputial Separation: Body weights, body weight gains, age of attainment of PPS and weight at day of PPS are presented in Table 3 below. Table 4 provides the proportion/incidence of unseparated. *(These tables are **MANDATORY**]*

TABLE 3. General Growth and Preputial Separation (PPS)[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 23; g)	U												
	A												
Body weight at PPS (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 PPS (PND)	U												
	A												

a Data were obtained from page [#] of the study report, and are the average of [#] male rats per dose group.
U = Unadjusted for body weight on PND 23
A = Adjusted for body weight on PND 23
SD = Standard Deviation
CV = Coefficient of Variation
* Significantly different from controls at $p<0.05$.
NA Not applicable

TABLE 4. Preputial Separation

	Vehicle Control	Low Dose ([#] mg/kg/day)	High Dose ([#] mg/kg/day)
Number of Animals Examined			
Incidence			

D. <u>Organ Weights</u>: Organ weights at necropsy are presented in Table 5 below.
*(This table is **MANDATORY**. Describe any treatment-related effects.)*

TABLE 5. Organ Weights at Necropsy[a]

Organ		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
Liver (g)	U												
	A												
	R												
Kidneys (g)	U												
	A												
	R												
Pituitary (mg)	U												
	A												
	R												
Adrenals (mg)	U												
	A												
	R												
Seminal vesicle + coagulating gland, with fluid (mg)	U												
	A												
Seminal vesicle + coagulating gland, without fluid (mg)	U												
	A												
Ventral prostate (mg)	U												
	A												
Dorsolateral prostate (mg)	U												
	A												
LABC (mg)	U												
	A												
Epididymis, left (mg)	U												
	A												
Epididymis, right (mg)	U												
	A												
Testis, left (mg)	U												
	A												
Testis, right (mg)	U												
	A												
Thyroid, fixed (mg)	U												
	A												

a Data were obtained from page [#] of the study report, and are the average of [#] male rats per dose group.
U = Unadjusted for body weight on PND 23
A = Adjusted for body weight on PND 23
R = Organ-to-body weight ratio (relative to body weight)
SD = Standard Deviation
CV = Coefficient of Variation
* Significantly different from controls at $p<0.05$.

E. <u>Clinical Chemistry and Hormone Levels</u>: 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)												
Serum testosterone (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 [#] male rats per dose group.
SD = Standard Deviation
CV = Coefficient of Variation
* Significantly different from controls at $p<0.05$.

F. <u>Histopathology</u>: 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 testes, epididymides and kidneys are presented in Table 8.

TABLE 8. Incidence of Histopathological Lesions of the Testes, Epididymides and Kidney[a]

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

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

III. DISCUSSION AND CONCLUSIONS

A. INVESTIGATOR'S CONCLUSIONS: *Provide a brief paragraph of the investigator's 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 preputial separation within acceptable ranges? Report findings relative to performance criteria?*
- *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 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.*