Appendix A8 – Pubertal Female

Pubertal Female	
Purpose	This assay is capable of detecting chemicals with estrogenic/anti-estrogenic activity, or agents which alter pubertal development via changes in steroidogenesis, or hypothalamic-pituitary regulation of the ovary and thyroid homeostasis.
Design	Test chemical is administered daily by gavage from post-natal day (PND) 22 to PND 42 (21 days) to 15 females per dose. Two doses plus vehicle control are employed. The animals are weighed daily, and examined for vaginal opening from PND 22 until opening is complete. After vaginal opening, vaginal smears are taken daily. Additional measures are taken at necropsy.
Endpoints	Growth (daily body weight) Age and weight at vaginal opening Organ weights: Uterus (blotted) Ovaries (paired) Thyroid Liver Kidneys (paired) Pituitary Adrenals (paired) Histology Uterus Ovary Thyroid (colloid area and follicular cell height) Kidney Blood Chemistry, standard panel Hormones Serum or plasma thyroxine (T_4), total Serum or plasma thyroid stimulating hormone (TSH) Estrus cyclicity Age at first estrus after vaginal opening Length of cycle Percent of animals cycling Percent of animals cycling regularly
Interpretation	Results are evaluated for evidence of interaction of the test chemical with the endocrine system, primarily estrogen- and thyroid-related. Body weight, organ weight, and hormone values for the control animals are subject to performance criteria for mean and coefficient of variation. Thyroid endpoints are generally interpreted separately from the sex-hormone-related endpoints.

Pubertal Female	
Main peer review comments	• On the whole, the purpose and protocol are clear • Vaginal opening is a sensitive endpoint for assessing estrogen function, alteration of steroidogenesis or HPG axis. • Uterine and ovarian weights in cycling animals are variable due to the estrous cyclicity and may not be useful endpoints. • The overall detection of the effects of the test chemicals was comparable across laboratories although not always on an endpoint by endpoint basis.
Strengths (within the context of the proposed battery)	• Intact mammalian *in vivo* system, and thus addresses ADME concerns. • Apical assay covering several modes of interaction, including ones not covered by other assays in battery • Redundant endpoints, maximizing chance for detection while minimizing false negatives • Covers pubertal period of development • Well-established relationship between endpoints and endocrine system
Limitations (within the context of the proposed battery)	• Protocol is not as diagnostic for specific MOAs as other assays in the battery such as uterotrophic for ER agonist. • Although a toxic negative chemical has not been identified, several chemicals positive for one of the MOAs have been found to be negative for the other MOAs evaluated in this assay.