Appendix A9 – Pubertal Male

Pubertal Male	
Purpose	Provide information obtained from an *in vivo* mammalian system that is useful in determining the potential of chemicals or mixtures to interact with the endocrine system. Detect chemicals with antithyroid, androgenic, or antiandrogenic [androgen receptor (AR) or steroid-enzyme-mediated] activity or agents which alter pubertal development via changes in gonadotropins, prolactin, or hypothalamic function.
Design	Test chemical is administered daily by gavage from post-natal day (PND) 23 to PND 53 (31 days) to 15 males per dose level at two dose levels plus vehicle control. The animals are weighed daily, and examined for preputial separation from PND 30 until separation is complete. The other measurements are taken at necropsy.
Endpoints	Growth (daily body weight) Age and weight at preputial separation Organ weights seminal vesicle plus coagulating gland ventral prostate dorsolateral prostate levator ani plus bulbocavernosus muscle complex epididymis testis thyroid liver kidney adrenal pituitary Blood Chemistry, standard panel Hormone levels serum testosterone, total serum thyroxine, total serum thyroid stimulating hormone Histology epididymis testis thyroid kidney

Pubertal Male	
Interpretation	Results are evaluated for evidence of interaction of the test chemical with the endocrine system, primarily androgen- 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 androgen-related endpoints.
Main peer review comments	• Assay is relevant to its purpose. • Hormone assays should be standardized and centralized QC standards maintained by EPA. [EPA will provide better guidance for standardization but will not maintain a centralized standard.] • Several endpoints are variable so the redundancy of endpoints is good.
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 elsewhere • Redundant endpoints, maximizing chance for detection while minimizing false negatives • Covers pubertal period of development • Well-established relationship between endpoints and endocrine system • Endpoints easy to measure
Limitations (within the context of the proposed battery)	• Variability of hormone measurements, particularly testosterone • Relatively long duration • 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.