Appendix C1 – Adult Male

Adult Male	
Purpose	To detect interactions with the endocrine system, especially chemicals that may be AR agonists/antagonists, steroid biosynthesis inhibitors, and gonadotropin and thyroid modulators either directly or indirectly through intact HPG or HPT axes. Versatility of the assay may also permit detection of potential ER agonists/antagonists, progesterone agonists/antagonists and prolactin modulators through neuroendocrine pathways.
Design	Adult male rats (~10 wks) are treated daily for 15 days by oral gavage at three dose levels (low, intermediate, high) plus a vehicle-control (0.25% methylcellulose) at a dose volume of 5 ml/kg. Dose concentrations (mg/kg/d) are adjusted daily based on body weight for all animals in all groups (n=15/group). On Day 15, final body weight is determined and animals are anesthetized and decapitated. Target organs and blood are collected within a 3 hour window during mid-morning.
Endpoints	Clinical observations, food consumption and body weight, daily Organ weights Liver Testes Epididymides Prostate (total) Seminal vesicles with coagulating gland containing fluid Accessory sex glands (prostate plus seminal vesicles with coagulating gland) Thyroid Hormone concentrations (assays may be run based on nature of test chemical and organ weight and histology results) Testosterone Dihydrotestosterone Estradiol Follicle-Stimulating Hormone Luteinizing Hormone Prolactin Thyroid-Stimulating Hormone Thyroxine Triiodothyronine Histology Testes Epididymides Thyroid

Adult Male	
Interpretation	Final body weight, organ weights (absolute and relative to final body weight) and hormone concentrations in the treated groups are statistically compared to those in the control group. A trend analysis is also done to determine the dose-response relationship for organ weights and hormone concentrations. Relevant historical control data may be used to further confidence in the performance of the bioassay results for organ weights and hormone concentrations in the vehicle-control group. Determination of whether the results in the treatment groups are endocrine-related first involves whether the final body weight decrement relative to the control group is within the limits of interpretation of an endocrine-related effect rather than an acute toxic effect secondary to an extreme decrease in final body weight during treatment. Primary effects associated with organ weights and histomorphology are assessed statistically (organ weights) and biologically (organ weights and histomorphology) to determine if there are endocrine-related responses due to treatment. Statistical and biological evaluations of hormone concentrations are used secondarily to support primary effects; they are not used alone within the bioassay. A weight-of-evidence approach with biological plausibility is considered among the multiple endpoints within the bioassay to conclude whether or not the bioassay has detected an interaction between the test chemical and the E, A or T hormonal pathways.
Main peer review comments	• Historical control data outdated and limited to one industrial laboratory • Within- and between-laboratory CVs for all endpoints were properly analyzed and relatively consistent for organ weight endpoints but highly variable for the hormonal endpoints • Running the full suite of hormonal assays is not justified • Hormonal assays were not standardized • Pre-validation studies involved extensive testing of a wide range of chemicals but inter-laboratory study involved too few chemicals covering a limited number of MOA (*i.e.*, anti-androgen and thyroid toxicant) • Negative or ambiguous results with relatively weak estrogenic and androgenic test compounds during pre-validation and inter-laboratory study

Adult Male	
Strengths	• Intact mammalian *in vivo* system taking into account ADME • Flexible to cover multiple MOA, receptor and non-receptor mediated • Dose setting is readily achieved without confounding factors (*e.g.*, growth and maturation of the HPG and HPT axes) • Short dosing duration of 2 weeks at 3 dose levels • Simple design comparable to sub-acute toxicology study • Multiple and complimentary male reproductive organs as primary endpoints with secondary hormonal endpoints; therefore, minimizing false negatives
Limitations or weaknesses	• Insensitive to relatively weak estrogenic and androgenic compounds • Extensive variability in hormone assay measurements, especially those relevant to androgen-dependent organs (LH, testosterone, DHT). • Hormonal results considered secondary that may or may not support primary organ weight and histological results.