Appendix A10 – Steroidogenesis (H295R)

Steroidogenesis (H295R)	
Purpose	Provides an *in vitro* cell-based assay to detect chemicals that affect the synthesis of the sex steroid hormones.
Design	H295R cells are incubated with 7 concentrations of test chemical in triplicate overnight at 37°C along with 2 concentrations of prochloraz and forskolin as positive controls.
Endpoints	17β- estradiol and testosterone content of the supernatant are analyzed using appropriate steroid hormone assays. Cell viability is measured by live/dead assay.
Interpretation	Final guidance for data interpretation will be provided in the integrated summary report. Currently fold induction or inhibition is the basis for expressing the outcome of the assay. The criteria for differentiating positive and negative outcomes will likely be minimum fold change, but may be statistically significant difference between controls levels
Main peer review comments	Peer review of this assay is expected in the spring of 2008.
Strengths (within the context of the proposed battery)	• Only *in vitro* assay that can evaluate effects on the entire steroidogenesis pathway—cells have all of the enzymes necessary for steroidogenesis. • Rapid and inexpensive • Detects chemicals that inhibit and induce steroidogenesis • Response is two dimensional (effective concentration and magnitude of response) and can distinguish among strong, moderate and weak inducers and inhibitors • Cells readily available from the ATCC

Steroidogenesis (H295R)	
Limitations (within the context of the proposed battery)	• Limited metabolism