Appendix A2 – Androgen Receptor Binding

Androgen Receptor Binding	
Purpose	The AR binding assay is a sensitive *in vitro* test to detect chemicals that may affect the endocrine system by binding to the androgen receptor. It will give added confidence that positive results seen in the Hershberger assay are truly due to an AR binding mechanism.
Design	Cytosol isolated from the rat prostate provides the source of the androgen receptor. Test chemical and R1881, a strong ligand, compete for binding with the AR when incubated together overnight. The assay measures the binding of [^{3}H]-R1881 in the presence of eight test chemical concentrations. Unlabeled R1881 serves as a strong positive control producing the standard curve. Dexamethosone is run with each block of test chemicals as a weak positive control. Solvent is the negative control.
Endpoints	The DPM of [^{3}H]-R1881 is measured by liquid scintillation counter. Data for the standard curve and each test chemical will be plotted as the percent [^{3}H] R1881 bound versus the molar concentration through use of a four parameter non-linear regression program.
Interpretation	Performance criteria have been set for the top, bottom, and slope for R1881 and the weak positive, dexamethasone. • If the binding curve crosses 50% (competes to displace the standard ligand by 50%) the test chemical is considered to be a binder. • Chemicals for which the binding curve crosses 75%, but not 50%, are considered to be equivocal. • Chemicals which do not fit the model or for which the binding curve does not cross 75% are considered to be non-binders.
Main peer review comments	• The AR assay is highly relevant to detect substances that bind to the AR receptor in humans. • Reproducibility of data for weaker chemicals by less proficient laboratories was a significant problem [EPA response: Performance standards have been developed that will screen out weaker laboratories.] • An adequate range and number of test chemicals were selected for validation • There are better assays for this purpose than this assay including recombinant assays and transcriptional activation assays. [EPA response: These other assays have not been validated and, therefore, are not ready for use in a regulatory program at this time.]

Androgen Receptor Binding	
Strengths (within the context of the proposed battery)	• Specific for identifying an interaction with the AR receptor providing mechanistic information • More sensitive than typical in vivo assays • Rapid • Inexpensive • It will give added confidence that positive results seen in the Hershberger and other male *in vivo* assays are truly due to an AR
Limitations (within the context of the proposed battery)	Although it detects both agonists and antagonists although it cannot predict the consequences of binding • Other steroids that are not natural ligands for the AR may bind at high concentrations • Chemicals that denature the receptor may be identified as false positives • No metabolic capability