Appendix A4 – Estrogen Receptor Binding

Estrogen Receptor Binding	
Purpose	The purpose of the estrogen receptor (ER) binding assay is to identify test chemicals that can bind to the estrogen receptor.
Design	A saturation radioligand binding experiment is conducted to demonstrate that the ER binding assay is working under optimal conditions within a given laboratory. This assay is conducted by measuring the equilibrium binding of increasing concentrations of ^{3}H-estradiol to rat cytosolic or human recombinant ERα. Nonlinear regression analysis of the data provides estimates of the affinity of the receptor for 17β-estradiol (K_d) and the concentration of receptors (B_{max}). A competitive ER binding assay is conducted by measuring the equilibrium binding of a single concentration of ^{3}H-17 β-estradiol at various concentrations (over a range of at least six orders of magnitude) of a test chemical in rat cytosolic or human recombinant ER. After equilibration, the amount of radioactivity bound to the ER is measured as an indicator of how much was displaced by the test compound at each concentration. Data analysis provides an estimate of the potency of the test chemical for binding to the ER relative to 17 β-estradiol. 17 β-estradiol is run as a reference standard with each run, as are a weak positive and a non-binder. In each portion of the study, three replicate data points are collected at each concentration in one run, and three independent runs are performed to constitute one assay.
Endpoint	Binding curve fit to a four-parameter Hill equation, where the parameters are top, bottom, slope, and log(IC_{50}) (*i.e.*, base-10 log of the molar concentration of test chemical which inhibits 50% of binding by the radioligand).
Interpretation	Performance criteria have been set for the top, bottom, and slope for 17β-estradiol and the weak positive, norethynodrel, for the competitive ER binding portion of the assay. Within-run variability is also subject to a performance criterion. Classification of test chemicals: Positive: A log(IC_{50}) value can be obtained from an unconstrained curve fitted to the Hill equation that has a slope of approximately -1.0. Equivocal: Acceptable binding curve reaches 25% displacement

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	of radioligand but not 50% at the highest concentration. Also applied if slope is unusually steep or shallow. Negative: Acceptable binding curve does not reach 25% displacement of the radioligand; or curve cannot be fit and no data point shows displacement of more than 25%.
Main peer review comments	• [Peer review of this assay expected by the end of August 2008.]
Strengths (within the context of the proposed battery)	• Quick (two days) • Uses relatively few, or no, animals (depending on source of receptor – rat uterine cytosol or human recombinant) • Specific for identifying an interaction with estrogen receptor (*i.e.*, provides mechanism-related information)
Limitations (within the context of the proposed battery)	• Although it detects both agonists and antagonists, it cannot distinguish the consequences of binding. • Does not account for potential metabolic activation or deactivation of test chemical.