Appendix A3 – Aromatase (Recombinant)

Aromatase (Recombinant)	
Purpose	The aromatase assay detects chemicals that inhibit aromatase activity. Aromatase is the enzyme that metabolizes androgens such as testosterone to estrogens
Design	Androstenedione and [1β-^{3}H]-androstenedione (ASDN) serve as substrate for human recombinant microsomal aromatase. Full activity control (ASDN in medium, no inhibitor), background activity control (no NADPH), positive control (4-hydroxyandrostenedione at eight concentrations) and test chemical (8 concentrations) are run in the reaction for15 minutes, and the reaction products produced are measured and plotted as percent enzyme activity (inhibition curve) through use of a non-linear regression program.
Endpoints	The formation of $^{3}H_2O$, one of the co-reaction products along with estrone, is measured by liquid scintillation counter.
Interpretation	Chemicals that reduce enzyme activity levels by 50% or more (as determined by the inhibition curve calculated by a four parameter non-linear regression program) are considered to be inhibitors of aromatase. Chemicals that fit the inhibition curve but allow 50-75% activity, *i.e.*, reduce activity by 25-50%, are considered equivocal. Chemicals that do not fir the model or that fit the model but reduce inhibition by 25% are considered to be non-inhibitors of aromatase.
Main peer review comments	• Comments supported the use of the assay for the intended purpose, the clarity of the protocol, the data interpretation procedure, and performance criteria; however, one reviewer noted that Ki determination would be superior to IC50. • The chemicals and analytical methods used in the validation of the assay were appropriately chosen. • The protocol could be further optimized for small volumes resulting in less cost and waste and greater convenience. [EPA response: This option will be permitted in the revised protocol.] • There are better assays that could have been selected such as cell-based assays which would be advantageous in that

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	they would detect both induction and inhibition. [EPA response: EPA is currently validating the H295R assay. See H295R fact sheet.]
Strengths (within the context of the proposed battery)	• Highly specific to inhibition of aromatase activity providing mechanistic information. • More sensitive than typical in vivo assays • Rapid • Inexpensive • Capable of high throughput • Provides useful information for the interpretation of in vivo assays.
Limitations (within the context of the proposed battery)	• Cannot detect chemicals that induce aromatase activity. • False positives could result from chemicals that denature the enzyme. • Limited/no ability to metabolize xenobiotics