Appendix A7 – Hershberger

Hershberger	
Purpose	A short-term in vivo assay to detect androgenic or antiandrogenic chemicals or chemicals that inhibit 5α-reductase.
Design	There are two versions of the Hershberger Assay: an immature version and a peripubertal version employing castrated rats. In addition, each version can be run to detect AR agonists or antagonists. When screening for potential androgenic activity, the test substance is administered daily by oral gavage or subcutaneous injection for a period of ten consecutive days. Test substances are administered to a minimum of two treatment groups of experimental animals using one dose level per group. When screening for potential antiandrogenic activity, the test substance is administered daily by oral gavage or subcutaneous injection for a period of ten consecutive days in concert with daily TP doses (0.2 or 0.4 mg/kg/d) by sc injection. Graduated test substance doses are administered to a minimum of two treatment groups of experimental animals using one dose level per group. In both the agonist and antagonist procedures, the animals are necropsied approximately 24 hours after the last dose.
Endpoints	The assay is based on statistically significant changes in weight in androgen dependent tissues. The five tissues weighed in the castrated male are ventral prostate (VP), seminal vesicle (SV) (plus fluids and coagulating glands), levator ani-bulbocavernosus (LABC) muscle, paired Cowper's glands (COW) and the glans penis (GP). In the immature version the GP cannot be detached and measured but the testes and epidimydes are weighed in the intact weanling.
Interpretation	A positive result is a statistically significant change in the weight of two of the tissues.

Hershberger	
Main peer review comments	• Assay could be used effectively to detect androgen agonists, antagonists and inhibitors of 5α-reductase. • Additional work should be undertaken to characterize the rate of false positives. [Addressed by OECD.] • Clear guidance should be provided on the MTD [Addressed by OECD in the test guideline.] • The data interpretation procedure needs further definition [Addressed by OECD in the test guideline.]
Strengths (within the context of the proposed battery)	• Relatively rapid screen that is quite specific to androgenic effects (see limitations) • In vivo procedure incorporates metabolism; thus, it can detect chemicals that need activation. • Oral administration will model a primary exposure route and incorporates ADME
Limitations (within the context of the proposed battery)	• The growth response of the individual androgen-dependent tissues is not entirely of androgenic origin, *i.e.,* compounds other than androgen agonists can alter the weight of certain tissues. However, the growth response of several tissues concomitantly substantiates a more androgen-specific mechanism.