

10.0 ANIMAL WELFARE CONSIDERATIONS

10.1 Refinement, Reduction, and Replacement Considerations

ICCVAM promotes the scientific validation and regulatory acceptance of new methods that refine, reduce, or replace animal use where scientifically feasible. Refinement, Reduction, and Replacement are known as the three Rs of animal protection. These principles of humane treatment of laboratory animals are described as:

- Refining experimental procedures such that animal suffering is minimized;
- Reducing animal use through improved science and experimental design; and
- Replacing animal models with nonanimal procedures (e.g., *in vitro* technologies), where possible.

Combes (2000) and Phillips (2000) recommended that adequate consideration be given to animal welfare concerns by careful development and validation of all proposed endocrine disruptor screening methods. With respect to the proposed use of *in vitro* AR TA assays as screening methods to detect substances that potentially exhibit androgenic or anti-androgenic activity, it is important to evaluate the current level of animal use in these assays, and to consider what opportunities exist for refining, reducing, or replacing procedures that use animals.

10.2 Use of Animals in *In Vitro* AR TA Assays

All 18 of the *in vitro* AR TA assays addressed in this BRD utilize cultured whole cells containing androgen-inducible gene expression systems and, therefore, do not require use of animals. Of these assays, the following use five different human cell lines that either naturally express hAR or are transfected with hAR vectors: 1) HeLa hAR(T)+Luc(T); 2) HepG2 hAR(T)+Luc(T)+ -gal(T); 3) MDA-MB453 hAR(E)+Luc(T)* (Transduced); MDA-MB453-kb2 hAR(E)+Luc(S); 4) PC-3 hAR(T)+Luc(T) and PALM hAR(S)+Luc(S); 5) LnCaP-FGC hAR(E)+CP. The first four assays require transfection with cDNA encoding the enzyme luciferase, which produces a luminescent signal that can be measured. The LnCaP-FGC+CP assay measures cell growth.

Four other groups of assays use mammalian cells from different species. One of these groups uses CV-1 monkey kidney cells that have been transfected with vectors encoding hAR and

luciferase or CAT (i.e., CV-1 hAR(T)+Luc(T)*; CV-1 hAR(T)+CAT(T); CV-1 hAR(T)+Luc(T)). Another assay uses this same cell line that is transiently transfected with vectors encoding mouse AR and the enzyme CAT (i.e., CV-1 mAR(T)+CAT(T)). A different group of assays uses CHO cells that have been transfected with vectors encoding hAR and luciferase or CAT (i.e., CHO hAR(S)+Luc(S); CHO hAR(T)+Luc(T); CHO-K1 hAR(S)+Luc(S); CHO-K1 hAR(T)+Luc(T)+EGFP(T); CHO hAR(T)+CAT(T)+ gal(T)). Another assay uses EPC (carp skin tumor) cells that have been transfected with vectors encoding rainbow trout AR (i.e., EPC rtAR (T)+CAT(T)).

The last group of assays uses stably transformed yeast cells containing cDNA for hAR (i.e., Yeast (*S.cer*) hAR(S) + -gal(S)) and measures the production of the enzyme -galactosidase.

From an animal welfare perspective, all of these *in vitro* cell-based assays are equally advantageous. However, because none of these assays has been extensively used for the routine testing of substances, further development and validation are required.