CHARGE TO PEER REVIEWERS
for
INDEPENDENT PEER REVIEW OF THE H295R CELL-BASED ASSAY FOR STEROIDOGENESIS
AS A POTENTIAL SCREEN IN THE ENDOCRINE DISRUPTOR SCREENING PROGRAM (EDSP) TIER-1 BATTERY

April 29, 2008

Background:

According to Section 408(p) of the EPA's Federal Food Drug and Cosmetic Act, the purpose of the EDSP is to:

> *develop a screening program, using appropriate validated test systems and other scientifically relevant information, to determine whether certain substances may have an effect in humans that is similar to an effect produced by a naturally occurring estrogen, or other such endocrine effect as the Administrator may designate [21 U.S.C. 346a(p)].*

Subsequent to passage of the Act, the EPA formed the Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC), a panel of scientists and stakeholders that was charged by the EPA to provide recommendations on how to implement the EDSP. Upon recommendations from the EDSTAC, the EPA expanded the EDSP using the Administrator's discretionary authority to include the androgen and thyroid hormone systems as well as wildlife.

One of the test systems recommended by the EDSTAC was the sliced testes assay. Its purpose in the Tier-1 battery was to provide a sensitive *in vitro* assay to detect chemicals that may affect the endocrine system by inhibiting the enzymes responsible for the inhibition of enzymes in the steroid hormone synthesis pathway. After encountering two substantial issues with the standardization of the sliced testes assay—high variability and the inability to distinguish general cytotoxicity from Leydig cell toxicity—EPA abandoned the sliced testes assay in favor of the H295R. The H295R assay offered a number of substantial advantages over the sliced testes assay and other cell-based assays. Like other cell based assays it does not use animal tissue and is capable of detecting inducers as well as enzyme inhibitors. Unlike the other cell-based assays, it contains all of the enzymes of the steroidogenic pathway.

Although peer review of the H295R assay will be done on an individual basis (i.e., its strengths and limitations evaluated as a stand alone assay), it is noted that the H295R assay along with a number of other *in vitro* and *in vivo* assays will potentially constitute a battery of complementary screening assays. A weight-of–evidence approach is also expected to be used among assays within the Tier-1 battery to determine whether a chemical substance has a positive or negative effect on the estrogen, androgen or thyroid hormonal systems.

The FIFRA Scientific Advisory Panel (SAP) has already conducted a peer review of the EPA's recommendations for the Tier-1 battery. The H295R assay was one of the assays

recommended by EPA contingent upon satisfactory validation and peer review of the assay.

This peer review will focus on the scientific work EPA performed to validate the H295R assay. Each peer reviewer is asked to focus his/her review on this issue. Unlike other peer reviews EPA did not have time to produce an Integrated Summary Report (ISR), so peer reviewers will be asked to focus on the interim final validation report and to a lesser extent on the prevalidation reports for conducting this review. It should be noted that in order to meet the August 2008 deadline for implementation, EPA is requesting review of the interlaboratory study on the 12 core chemicals and the 18 supplementary chemicals that were tested in the lead laboratory. When the other participating labs have completed testing of the 18 supplementary chemicals a final report will be prepared which will also undergo peer review.

Charge Questions:

Your review and comments should be directed to each of the following questions:

1. Is the stated purpose of the assay clear?
2. Is the assay biologically and toxicologically relevant to the stated purpose?
3. Does the protocol describe the methodology of the assay in a clear, and concise manner so that the laboratory can:
    a) comprehend the objective;
    b) conduct the assay;
    c) observe and measure prescribed endpoints;
    d) compile and prepare data for statistical analyses; and
    e) report the results?
    What additional advice, if any, can be given regarding the protocol?
4. Have the strengths and/or limitations of the assay been adequately addressed?
5. Were the (a) test substances, (b) analytical methods, and (c) statistical methods chosen appropriate to demonstrate the performance of the assay?
6. Considering the variability inherent in biological and chemical test methods, were the results obtained with this assay sufficiently repeatable and reproducible?
7. With respect to performance criteria, were appropriate parameters selected and reasonable values chosen to ensure proper performance of the assay?
8. Are the data interpretation criteria clear, comprehensive, and consistent with the stated purpose?
9. Please comment on the overall utility of the assay as a screening tool in the EDSP Tier 1 battery.