PEER REVIEW CHARGES

for

INDEPENDENT PEER REVIEW OF THE FEMALE PUBERTAL RAT ASSAY AS A POTENTIAL SCREEN IN THE ENDOCRINE DISRUPTOR SCREENING PROGRAM (EDSP) TIER-1 BATTERY

October 1, 2007

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 female pubertal rat assay. The purpose of the pubertal assay is to provide information obtained from an *in vivo* mammalian system that will be useful in assessing the potential of a chemical substance or mixture to interact with the endocrine system. This assay is capable of detecting chemicals with antithyroid, estrogenic, or antiestrogenic [estrogen receptor (ER) or steroid-enzyme-mediated] activity or agents which alter pubertal development via changes in gonadotropins, prolactin, or hypothalamic function.

Briefly, the study design uses weanling rats, standardized to 8 - 10 per litter at post-natal day (PND) 3-5, that are housed 2 to 3 per cage. The test chemical is administered in corn oil by oral gavage (2.5 to 5.0 ml/kg) between 0700 and 0900 (lights 14:10, on 0500h) from PND 22 - 42 (21 days) to 15 females per dose level. The endpoints are growth (body weight); age and weight at vaginal opening; organ weights (uterus, blotted; ovaries; thyroid; liver; kidneys; pituitary; adrenals); histology of uterus, ovary, thyroid, kidney; serum thyroxine, total; serum thyroid stimulating hormone; age at first estrus, length of estrous cycle, percent of animals cycling, and percent of animals cycling regularly.

Although peer review of the female pubertal assay will be done on an individual basis (i.e., its strengths and limitations evaluated as a stand alone assay), it is noted that this 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 expected to be used among

assays within the Tier-1 battery to determine whether a chemical substance interacts with the endocrine system. Peer review of the EPA's recommendations for the Tier-1 battery will be done at a later date by the FIFRA Scientific Advisory Panel (SAP).

Each peer reviewer is asked to review the Integrated Summary Report and protocol (Appendix 1), and comment on the results of the validation process of the female pubertal rat assay, especially the inter-laboratory validation exercise[1]. Review and comment shall be directed to each of the following:

1. Clarity of the stated purpose of the assay.

2. Clarity, comprehensiveness and consistency of the data interpretation wit the stated purpose of the assay.

3. Biological and toxicological relevance of the assay as related to its stated purpose.

4. Clarity and conciseness of the protocol in describing the methodology of the assay such 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 results.

5. Strengths and/or limitations of the assay in the context of a potential battery of assays to determine interaction with the endocrine system.

6. Impacts of the choice of:
    a. test substances,
    b. analytical methods, and
    c. statistical methods in terms of demonstrating the performance of the assay.

7. Repeatability and reproducibility of the results obtained with the assay, considering the variability inherent in the biological and chemical test methods.

[1] The other Appendices are provided for your information, not necessarily for review. Appendix 2, the Critical Reviews in Toxicology paper that serves as the Detailed Review Paper of the basis for the assay may be of particular interest since it contains most of the information pertaining to relevance of the assay to the goal of Tier 1 assays, which is to determine whether a chemical has the potential to interact with the endocrine system.