CHARGE TO PEER REVIEWERS
for
INDEPENDENT PEER REVIEW OF THE ESTROGEN RECEPTOR BINDING ASSAY AS A POTENTIAL SCREEN IN THE ENDOCRINE DISRUPTOR SCREENING PROGRAM (EDSP) TIER-1 BATTERY

March 13, 2009

Background:

Section 408(p) of the Federal Food Drug and Cosmetic Act requires the EPA 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)].*

EPA established the Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC), a panel of scientists and stakeholders, to provide recommendations on how to implement the Endocrine Disruptor Screening Program (EDSP). EDSTAC recommended expanding the EDSP beyond the estrogens to include the androgen and thyroid hormone systems, and beyond humans to include wildlife. EDSTAC also recommended that several assays be included in a Tier 1 battery of assays to determine the potential of a substance to interact with the endocrine system, and several additional assays to be included in a Tier 2 battery of assays to confirm interaction, establish whether adverse effects may occur, and examine the dose-response relationship.

One of the test systems recommended by the EDSTAC for the Tier 1 battery was the estrogen receptor (ER) binding assay. Its purpose in the Tier-1 battery is to detect chemicals that may affect the endocrine system by binding to the ER. EPA requested the National Institute of Environmental Health Sciences (NIEHS) to prepare a comprehensive historical review and critical evaluation of ER binding assays that had been reported in the scientific literature. According to the Expert Panel convened by NIEHS which examined the review, no existing ER binding method was adequately detailed and standardized to be considered "validated". The Expert Panel recommended that the EPA focus its attention on validating an ER binding assay that uses recombinant receptor rather than receptor obtained from whole animals (rat uterine cytosol, RUC). The EPA subsequently initiated such a validation effort for a human recombinant ER (hrER) binding assay, but felt that it had already invested so much time and effort in validating the ER-RUC assay that it would be appropriate to complete the validation of that assay as well. The current peer review focuses on the ER-RUC assay; peer review of the hrER assay will be conducted separately since validation of that assay is running somewhat behind that of the ER-RUC assay.

Although the ER binding assay, like all of the assays in the Endocrine Disruptor Screening Program, will be peer reviewed separately from the other assays in the program, it is expected to be used in conjunction with other assays to determine the potential of a chemical to interact with the endocrine system. This "battery of assays"

approach was designed by EDSTAC to take advantage of the unmediated sensitivity of *in vitro* assays and other pathway-specific assays, while at the same time incorporating whole-animal assays that can detect effects that may be due to metabolites and some of which can detect effects from several different endocrine-related pathways. A weight-of–evidence approach using information from all of the assays, not just the ER binding assay, will be used to determine whether a chemical substance has the potential to interact with the endocrine system.

This peer review should focus on the strengths and weaknesses of the ER binding assay itself, not on the Integrated Summary Report *per se* or any of the individual studies that were conducted as part of the validation of the ER binding assay.

Charge Questions:
Please respond 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 described in the introduction of the ISR to be used by the EPA to identify chemicals that have the potential to interact with the endocrine system.