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

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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 Amphibian Metamorphosis Assay (AMA). The AMA consists of multiple endpoints; principally, developmental stage, hind limb length, body length (whole body and snout-vent), histology of the thyroid glands, mortality and morbidity. It is intended to empirically identify substances which may interfere with the normal function of the hypothalamic-pituitary-thyroid (HPT) axis. It represents a generalized vertebrate model to the extent that it is based on the conserved structure and functions of thyroid systems. It is an important assay in the EDSP screening battery because amphibian metamorphosis provides a well-studied, thyroid-dependent process which responds to substances active within the HPT axis, and it is the only assay in the battery that assesses thyroid activity in an animal undergoing morphological change. The AMA is not intended to quantify or confirm endocrine disruption, or to provide a quantitative assessment of risk, but only provide suggestive evidence that thyroid regulated processes may be sufficiently perturbed to warrant more definitive testing. A weight-of-evidence approach among the multiple endpoints within the bioassay, combined with biological plausibility, is expected to help distinguish endocrine-related effects from spurious effects and to determine whether a chemical substance has a positive endocrine effect on the HPT axis.

Although peer review of the AMA will be done on an individual basis (i.e., its strengths and limitations evaluated as a stand alone assay), this assay, along with a number of other *in vitro* and *in vivo* assays, will likely constitute a battery of complementary screening assays. A weight-of–evidence approach will also be used *among* assays within the Tier-1

battery to determine whether a chemical substance has the potential to interact with the endocrine system and whether Tier-2 testing is necessary. Peer review of the EPA's recommendations for the Tier-1 battery will be performed at a later date by the FIFRA Scientific Advisory Panel (SAP). The battery peer review will focus, in part, on the issue of coverage of known modes of endocrine activity, and how well individual assays work in concert with one another within the Tier-1 battery. Hence, it is important to peer review each individual assay.

Each peer reviewer is asked to review the Integrated Summary Report and draft test method, with accompanying support materials, and comment on the results of the validation process of the AMA, with special attention given to the inter-laboratory validation exercise. 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 with 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 test method in describing the methodology of the assay such that a 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.

    If warranted, please also make suggestions or recommendations for test method improvement.

5. Strengths and/or limitations of the assay.

6. Impacts of the choice of test substances and methods chosen to demonstrate 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.

8. Please comment on the overall utility of the assay as a screening tool, to be used by the EPA, to identify chemicals that have the potential to interact with the endocrine system sufficiently to warrant further testing.