ICCVAM Comments on OECD Draft Updated Test Guideline 407

General Comment:

The draft Test Guideline (TG) states that the rationale for updating the OECD TG 407 (*Repeated Dose 28-Day Oral Toxicity Study in Rodents*) with a number of endocrine and reproductive end points is to serve as an *in vivo* method providing data about multiple endocrine mechanisms and effects. The draft TG further states that the results from any testing using the updated OECD TG 407 should be considered in the context of the OECD Conceptual Framework for the Testing and Assessment of Endocrine Disrupting Chemicals. In this Conceptual Framework TG 407 is contained in Level 4. In general, we question the validity of this rationale and the appropriateness of including TG 407 in the OECD Conceptual Framework. This is supported by the comments that follow.

The rationale for the update was based on assessments made in the late 1990's, which concluded that there were no existing TG's that could adequately detect endocrine active substances (EAS). At the time of this initial assessment, it was reasonable to propose that adding endocrine and reproductive endpoints to the existing TG 407 could help fill this gap right away, especially since TG 407 is routinely required to fulfill regulatory data requirements because of the broad spectrum of endpoints and observations that are included. However, considering the OECD activity focused on the validation of *in vitro* and *in vivo* test methods that are designed to screen for EAS with considerably more specificity and sensitivity than the updated TG 407, the original rationale would seem outdated. Importantly, most if not all of the alternative EAS screening test methods will be included in Levels 2 or 3 of the OECD Conceptual Framework. Therefore, it is difficult to understand what value would be added by testing potential EAS at Level 4 with a less sensitive assay, i.e., TG 407. This is further supported by the explicit admission in the draft TG that the updated 407 test method should not be considered to be a broad based screening assay for endocrine activity because it could not detect weak EAS leading to a high rate of false negatives. Furthermore, the updated TG 407 may also lead to a high rate of false positives because it can not differentiate between receptor mediated effects and other toxic effects that may cause changes in reproductive organs (e.g., changes to the uterus could be estrogen receptor mediated, or from metabolic changes resulting in decreased or increased circulation of 17β-estradiol, or caused by other unknown mechanisms of toxicity).

Comments on Validation Studies:

OECD test guidelines should be based on adequately validated test methods (OECD GD 34), including the appropriate demonstration of intralaboratory reproducibility. There is no clear indication from the OECD Validation Report (OECD Series on Testing and Assessment 59) that any substances were tested independently more than once in any of

the participating laboratories. Therefore, extent of reproducibility of the test method within laboratories over time has not been demonstrated.

Also of concern in the validation studies is the use of uncoded test substances. Considering the importance of the histopathological examination of reproductive tissues in the updated TG, prior knowledge of test substance identity could potentially effect the objectivity of individual pathologists and therefore, further compromise the validation effort for this test method.

Lastly, all validation studies were conducted with rats only. Therefore, unless bridging studies are conducted with mice, all reference to the term "rodents" in the TG should be changed to "rats."

Technical Comments:

We concur with the technical comments made by the Peer Review Panel provided in the OECD Summary Report of the Peer Review Panel for the Updated TG 407 with the following additions:

- Serum and plasma hormone measurements in animals with such a small group size (n=5) is problematic, particularly with those that may have cyclic patterns and marked diurnal variation. This is acknowledged in the TG with specific regard to thyroid hormones (para 1f) but could equally apply to reproductive hormones.

- In the female, estrous cyclicity evaluations are conducted for five consecutive days at the end of the study. This is an important addition in determining when the females should be killed to aid in the evaluation of the reproductive tract (and particularly the uterus). However, since most rats are on a four or five day cycle this will provide no information on the potential endocrine effects on the hormonal control of cyclicity (as noted in para 23b). Thus, no indications of irregularity, extended periods in different phases, etc., can be garnered and information gained on potential effects on the female endocrine axis is extremely limited.

- The TG could also provide more guidance on how histopathology of the reproductive organs should be conducted. For example, in the male, reference could be made to appropriate fixation of the testis (the use of Bouin's solution has become an issue in many laboratories because of the use of the explosive picric acid) and its evaluation (see Creasy 2003; Ladtendresse et al. 2002).

References:

Creasy, DM, 2003. Evaluation of testicular toxicology: a synopsis and discussion of the recommendations proposed by the Society of Toxicologic Pathology *Birth Defects Res B Dev Reprod Toxicol* **68**, 408-15.

Ladtendresse, JR, Warbrittion, AR, Jonassen, H, and Creasy, DM, 2002. Fixation of testes and eyes using a modified Davidson's fluid: comparison with Bouin's fluid and conventional Davidson's fluid. *Toxicol Pathol* **30**, 524-33