

4/27/2004

To: Jane Smith

From: Jim Stevens

Subject: Response to the EPA question:

EPA Questions for the EDMVAC April 26-28, 2005

Questions for the Steroidogenesis Assay.

1. Should we proceed with validation or are additional studies necessary before validation can begin? If so, what does the Committee recommend?

The sliced testes assay would appear to have the major issues. The most important and central to its utilization is the small percentage steroid metabolizing cells in the total preparation; this contributes to high level of variability associated with this screen and clearly reduces its value. Further a satisfactory tool for estimating cytotoxicity has not been developed and does not appear to be forthcoming. Therefore, the validation effort for this screen should not proceed, and it is recommended that this screen should be abandoned for a more specific assay capable of measuring the ability to affect steroidogenesis. The alternative screen (H295R adrenal cancer cell model) would at first blush appear to be a less problematical system. As this model is an aberrant immortalized cancer cell line, it may not be reliable for providing answers in regard to what would occur in the native human adrenal cell. However, it still might be acceptable as a screen and be significantly easier to validate. Some effort should be taken by the EPA to identify another alternative model for the steroidogenesis assay.

Questions for the Uterotrophic Assay.

Many of the considerations involved in the design and execution of the validation of the uterotrophic assay are similar to other *in vivo* assay validations. While most of the other *in vivo* assays are not considering several protocols as they proceed to validation, the question of which experimental conditions to standardize (e.g., strain, feed, bedding, etc.) and the number and nature of the reference chemicals to use has been raised with several assays. Thus, the ultimate conclusions as to the adequacy of the validation of the uterotrophic assay may set a precedent for the remaining EDSP assays as they continue through validation and peer review. The EPA is requesting that the Endocrine Disruptor

Methods Validation Advisory Committee (EDMVAC) also examine the issues raised by the peer review and provide their opinion(s) on the adequacy of the validation process.

As other members of the committee have already mentioned (via correspondence), it is not possible to address the issues raised by the peer review as it is not yet available for the EDMVAC to review. However, it is clear the results from the great effort and resource expended to-date for the validation Uterotrophic Assay as indicated by the OECD Phase 2 Testing that the protocols are robust and reliable for identifying estrogen agonists, and the screen is transferable across laboratories. If possible there should be a narrowing of protocol options available. The immature female with 3-days peroral dosing, or option A, would appear to be the most palatable to all stakeholders, despite the OECD mandate. Further, positive and negative controls should be added to standardized protocol.

The Agency should consider more effort to validate the estrogen and androgen receptor binding screens which would provide a less expensive alternative to the Uterotrophic assay which would be less animal use intensity but provide similar screening information.

Questions for Fish Short Term Reproduction Assay.

The fish short term reproduction assay is intended to be used in the Endocrine Disruptor Screening Program Tier 1 screening battery to capture estrogen and androgen active substances and provide presumptive evidence to trigger Tier 2 definitive testing of potential adverse effects.

1. For the fathead minnow, are the available data adequate to demonstrate that the assay is capable of responding to estrogen and androgen active substances? If not, what more is recommended?

The 21-day screen in the fathead minnow appear to be responsive to both weak and strong estrogen and androgen antagonists.

Primary endpoints considered for inclusion consist of vitellogenin, secondary sex characteristics, gonad histopathology, and fecundity. 2. Should additional endpoints be considered for purposes of screening potential endocrine disrupting substances?

The primary endpoints of vitellogenin induction and secondary sex characteristics appears to work as a type 1 screen for detecting estrogenic and androgenic substances some strains of fish (primarily fathead minnows and medaka). Fecundity may be for an internal QC component but is not essential for the screen; however, it would appear that histopathology, in these species, is not ready for primetime. Furthermore, histopathology may be too expensive to be used for screening purposes. The male fat pad may be an additional endpoint measurement endpoint that might be added.

2. Given that the fathead minnow is a species of interest and fecundity is an endpoint of interest, what would constitute a “false positive” in terms of a response observed in the assay and warranting Tier 2 definitive testing? How should “false negatives” be addressed?

One way to establish false positive rates in the fathead minnow screen would be to run more materials known to be devoid of estrogenic or androgenic properties through the assay.

3. What additional data are recommended to demonstrate the validity of the screening assay for capturing potential adverse effects and triggering Tier 2 testing?

The 21-day fish reproduction assay, as proposed, includes apical endpoints (fecundity and histopathology) that may be confounded by toxicity mechanisms other than estrogenic and androgenic. As indicated above fecundity might be used for QC purposes; histopathology should be dropped from the screen.

Questions for the Amphibian Metamorphosis Assay.

The amphibian metamorphosis assay is intended to be used in the Endocrine Disruptor Screening Program Tier 1 screening battery to capture thyroid active substances and provide presumptive evidence to trigger Tier 2 definitive of potential adverse effects.

1. Given the planned OECD Phase 2 validation work, what additional data are recommended, if any, to demonstrate the validity of the screening assay for capturing presumptive thyroid active substances and triggering Tier 2 testing?

Continue to focus on thyroid histology and standardizing histopathology of the thyroid gland as the main endpoint. Besides other chemicals than thyroid stimulating chemicals, including negative chemicals, weak antagonist should be tested in the model.

Because the frog metamorphosis screen was added as a surrogate screen for evaluating the effects of chemicals on the thyroid, when the model has completed its Phase 2 testing, the agency should perform a comparison of frog screen with the pubertal male and female screen as far as their ability to detect agents affecting the thyroid. It is obvious that that the mammal would also be a preferred model to determine potential effects on the human thyroid. The modified 407 being developed in Europe and intact male models may provide further alternative screens for examining thyroid effects.