UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY AND POLLUTION PREVENTION

MEMORANDUM

Date: March 16, 2011

SUBJECT: Response to Technical Questions from Huntingdon Life Sciences Regarding Conduct of the EDSP Aromatase Assay Using Guideline 890.1200.

PC Code: NA
Decision No.: NA
Petition No.: NA
Risk Assessment Type: N/A
TXR No.: NA
MRID No.: NA

DP Barcode: NA
Registration No.: NA
Regulatory Action: NA
Case No.: NA
CAS No.: NA
40 CFR: NA

FROM: Greg Akerman, Ph.D.
Executive Secretary
Endocrine Disruptor Review Team

THROUGH: Karen Whitby, Ph.D., Co-Chair
Endocrine Disruptor Review Team
Office of Pesticide Programs
And
Les Touart Co-Chair
Endocrine Disruptor Review Team
Office of Science Coordination and Policy

TO: Richard Keigwin, Director
Pesticide Re-evaluation Division

CONCLUSION

The Endocrine Discuptor Review Team has provided responses to several technical questions received from Huntingdon Life Sciences regarding the conduct of EDSP 890.1200 Aromatase Assay.

I. ACTION REQUESTED

In response to the request received from Anne Matthews of Huntingdon Life Sciences, the Endocrine Review Team has been requested to provide a generic response to technical questions regarding the conduct of the EDSP Guideline 890.1200 Aromatase assay.

II. BACKGROUND

The Agency formed the Endocrine Disruptor Review Team (EDRT) to support OCSPP scientists and the regulated community in the review and conduct of the EDSP Tier 1 battery and requests for the use of alternate test protocols that may be requested by Test Order recipients or the public in response to EDSP Tier 1 test orders.

III. AGENCY'S RESPONSE TO TECHNICAL QUESTIONS

In e-mails dated February 9, 2011 and February 17, 2011, Anne Matthews of Huntingdon Life Sciences stated that she had "successfully completed the validation of the aromatase assay with regard to carrying out three runs with each of the four proficiency chemicals compared with the positive control chemical. However since starting to use the assay for a study with a test chemical she encountered several problem", *viz*:

1. The full activity controls at the beginning of the assay have been consistently markedly higher than those at the end. Initially I (Huntingdon) thought this may have been due to using DMSO as the solvent whereas during the validation process all of the compounds were soluble in ethanol. However, further experiments suggest this is not the case. I (Huntingdon) have noted that this phenomenon was mentioned in the Integrated Summary Report on Aromatase (2007) but the magnitude of the effect (described in several places as 'statistically significant') and a means of resolving it was not.
2. Whilst carrying out the entire validation process I (Huntingdon) used Supersomes™ from this 2nd lot had a lower initial aromatase activity. It is possible, although I (Huntingdon) cannot be certain, that the lower activity Supersomes™ lose activity more rapidly through a run. Do you know whether any of the laboratories that participated in the initial comparison process (or others subsequently) have experienced similar problems?
3. In addition, when validating the assay with the proficiency chemicals I diluted the Supersomes™ to a lesser degree than stated in the Guideline and then added only 50 µL to the tubes in the water bath, having kept the Supersomes™ on ice. However, when starting the study I (Huntingdon) diluted the Supersomes™ as per the methodology in the guideline and added them to the water bath at the same time as the 1st tube, prior to starting the reaction with 1 m? of these more diluted Supersomes™. Since the Supersomes™ have been diluted in the assay buffer and the final protein concentration is the same, would it be acceptable to add 50 µL of less diluted Supersomes™, kept on ice, to the assay, having previously added a total volume of 1.95 mL of components to the assay tubes?
4. When validating the assay I (Huntingdon) decided to remove the upper (aqueous) layer following the 1st two extraction procedures and transfer this to a fresh tube each time

prior to the addition of further aliquots of methylene chloride. This seemed to be more sensible, as it posed less risk of contamination of the aqueous layer compared with a pipette being inserted into the lower (methylene chloride) layer in order to remove this solvent, as is suggested in the Guideline. I (Huntingdon) have made the assumption that this will be acceptable to the EPA as the positive control and the proficiency chemicals tested during the validation gave similar results to those stated in the Guideline. Please could this be confirmed?

5. We have also investigated the use of propylene glycol in the assay, since this issue was raised in the Aromatase Assay Peer Review document. With regard to this, it was found that the activities seen were very similar, if not marginally greater, without the use of propylene glycol than those observed when it was added. Since slightly amending the assay conditions has increased the activities at both the beginning and the end of a run, would the EPA consider that these are valid variations to the CYP aromatase assay?

IV. AGENCY'S RESPONSE

The alterations to the testing protocol for the Aromatase Assay are relatively minor and are technically sound. However, without having the opportunity to review the actual data from the studies the Agency is unsure how the data from their assay will compare with the Aromatase Assay 890.1200 Guideline performance criteria. Any changes will require documentation along with actual data to demonstrate that performance criteria and other considerations have been met. The Agency fully recognizes that departure from the recommendations in the guideline may be appropriate in specific situations. Since Huntingdon has experienced improved assay results (e.g., higher maximal enzymatic activity; retention of maximal activity at beginning and end of the run) in their laboratory following the change in the dilution/warming procedure, it is appropriate to propose this alternative for conducting the assay in your laboratory.

The minor changes proposed are technically sound since the concentration of the enzyme (Supersomes^{TM}) is retained, and with the addition of the smaller, more concentrated aliquot of enzyme to the reaction mixture, the needs for pre-warming the Supersomes^{TM} appears to be no longer necessary. However, as noted in the test guideline, any changes should be well documented and supporting data will need to be submitted to the Agency to demonstrate the performance criteria (including enzymatic activity, positive controls, and proficiency chemicals are being met. If the Agency can provide further assistance, please contact us.