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

OFFICE OF CHEMICAL SAFETY AND POLLUTION PREVENTION

MEMORANDUM

DATE: **June 16, 2011**

SUBJECT: **Responses to EDSP Tier 1 Technical Questions Received from CeeTox**

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

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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, Ph.D., Co-Chair
Endocrine Disruptor Review Team
Office of Science Coordination and Policy

TO: Richard Keigwin, Director
Pesticide Re-evaluation Division

CONCLUSION

In response to the request received from Colleen Toole, Director of Project Management of CeeTox, the Endocrine Disruptor Review Team (EDRT) has provided responses to questions regarding assessing false positives in the Estrogen Receptor Transcriptional Activation (ERTA) Assay.

I. ACTION REQUESTED

Provide responses to questions received from CeeTox regarding the conduct of the Estrogen Receptor Transcriptional Activation (ERTA) 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 made by Test Order recipients or the public in response to EDSP Tier 1 Test Orders.

The Agency received an email from CeeTox (contract laboratory) requesting clarification on the information provided on page A-3 of the Test Guideline for the Estrogen Receptor Transcriptional Activation (ERTA) Assay on assessing false positives.

III. AGENCY's RESPONSE TO TECHNICAL QUESTIONS

In an email dated May 10, 2011, Colleen Toole of CeeTox requested clarification from the Agency for the following:

> "We have been testing chemicals in the ERTA assay (OPPTS 890-1300) using the Hela-9903 cell line as per the guideline. On A-3 of the guideline, there is a brief description of assessing false positives. The guideline appendix is very vague and does not cover very much information, nor was this part of the Pre-validation and Inter-laboratory Validation issued in 2006 by Masahiro Takeyoshi.
>
> It is not clear if this is part of the testing-
> If it is part of the testing is this assay to be completed for only ERTA positive test substances?
> How many times should this assay be performed? Are there any performance criteria?
> This does not seem to be part of the OECD guideline?
>
> I would appreciate any insight into how we should proceed for testing"

Agency Response:

The purpose of the information found in Appendix 2 (page A-3) of the guideline for OPPTS 890.1300: Estrogen Receptor Transcriptional Activation Assay is to discuss possible interactions that could result in a false positive in this assay and to suggest what to do if you suspect you have a false positive response. False positives may result from an increase in luciferase activity or fluorescence unrelated to interactions with the estrogen receptor (ER).

The procedure described in Appendix 2 is *not* required for every positive response. Appendix 2 is optional and may be performed if the laboratory suspects that the test material is exhibiting a false positive response in the ERTA assay. If a laboratory chooses to test a compound for a false positive response, the Agency recommends an approach similar to that used for testing chemicals in the ERTA assay, *i.e.*, perform the test in 2 or 3 replicate assays. There are no specific performance criteria for testing for false positives.

The information in Appendix 2 of OPPTS 890.1300 is also found in ANNEX 2 (page 15) of the OECD Guideline Test No. 455: The Stably Transfected Human Estrogen Receptor-alpha Transcriptional Activation Assay for Detection of Estrogenic Agonist-Activity of Chemicals (DOI : 10.1787/9789264076372-en).

IV. CONCLUSION

In response to the request received from Colleen Toole, Director of Project Management of CeeTox, the Endocrine Disruptor Review Team (EDRT) has provided responses to questions regarding assessing false positives in the Estrogen Receptor Transcriptional Activation (ERTA) Assay.