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

**OFFICE OF CHEMICAL SAFETY AND POLLUTION PREVENTION**

**MEMORANDUM**

**DATE:** **March 15, 2011**

**SUBJECT:** **Responses to EDSP Technical Questions Received from CeeTox Contract Laboratory**

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

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**Regulatory Action:** N/A
**Case No.:** N/A
**CAS No.:** N/A
**40 CFR:** N/A

**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 Cee Tox, the Endocrine Disruptor Review Team (EDRT) has provided responses to several generic technical questions regarding the conduct of *in vitro* EDSP Tier 1 assays.

## I. ACTION REQUESTED

Review generic technical questions received from CeeTox regarding the conduct of *in vitro* EDSP Tier 1 assays.

## 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.

## III. AGENCY's RESPONSE TO TECHNICAL QUESTIONS

**Question 1**. For oily compounds how do we ensure (or do we?) that the test article is exposed to the cells and not sitting on top of the media in the exposure plates? We use a nephelometer to look at solubility of the compounds in our media (identical plate to exposure plate), however with oils often insolubility is not picked up (except at high concentrations) by light scatter. I am concerned that the test article would give a false negative as exposure to the cells might be limited, or non-existent.

**Response 1**. Guidance for determining limit solubility concentrations has been provided in the test guidelines. In cases such as you mention, some labs have successfully used a low power light microscope to detect issues with chemical solubility.

**Question 2**. If we do have an oily compound, what limitations do we have for increasing exposure to the cells? One of our thoughts is to examine microemulsions of the compound (SMEDDs) which is one method of increasing bioavailability (I have several papers I could send reviewing SMEDDs). Many of the *in vitro* guidelines only mention DMSO or EtOH as a vehicle, however this will be an issue for many of our clients test articles.

**Response 2**. Due to tremendous diversity in chemical structure/physical properties among chemicals under EPA's purview, it is likely that some chemicals will not be amenable for evaluation in all *in vitro* assays. However, if after attempts using due diligence and good laboratory practices (GLP), a chemical is believed to be incompatible with a particular *in vitro* assay, the results/information should be well documented (with data) and submitted to the Agency for consideration. If the Agency agrees, the chemical will be classified as incompatible with that assay/test system. In addition, the Chemical Review Manager in the Pesticide Re-evaluation Division of OPP can be contacted if further guidance is needed prior to submitting data to the Agency. Physical chemical characteristics of some test materials may limit which Tier 1 screens can actually be conducted; to address such cases, the Agency included both *in vitro* and *in vivo* screens in the Tier 1 battery assays with complementary endpoints.

**Question 3**. How do we handle waxy compounds (same issues as above)?

**Response 3**. Please see response to question number 2 above.

**Question 4 a)** For test articles that are in another vehicle, do we run the alternative vehicle control with the reference compounds in addition to the reference compounds in DMSO?

**Response 4a)** Yes, to demonstrate new vehicle is comparable to DMSO.

**Question 4b)** The reference controls in DMSO would provide information that the assay is working and within the guidelines, and the reference controls in the alternative vehicle would let us know if there was any effect of the vehicle on the reference controls. This expands out the testing for the in vitro assays significantly (4 reference controls now become 8). If the vehicle does have an effect on the reference controls is the test article then considered incompatible with this assay?

**Response 4b)** Test guidelines are intended to provide guidance and recommendations for conducting the assay, and although the procedure is strongly recommended for generating the data that are the subject of this guideline, EPA recognizes that departures may be appropriate in specific situations. However, as noted in each test guideline, any changes should be well documented and supporting data will need to be submitted to EPA to demonstrate that performance criteria (including solvent, positive and negative controls; all reference/proficiency chemicals) are being met.

An inability to demonstrate that an alternate vehicle is comparable with that of DMSO would be of significant concern. This would be a case where the lab should contact the Agency's Chemical Review Manager for further guidance.

**Question 5**. If there is significant interference by the test article in the assay, is the test article then considered incompatible with the assay?

**Answer 5**. Due to tremendous diversity in chemical structure/physical properties among chemicals under EPA's purview, it is likely that some chemicals will not be amenable for evaluation in all *in vitro* assays. However, if after attempts using due diligence and good laboratory practices (GLP), a chemical is believed to be incompatible with a particular *in vitro* assay, the information should be well documented (with data) and submitted to the Agency for review and consideration. If there are additional question the Chemical Review Manager should be contacted for further guidance. It is for this reason that both *in vitro* and *in vivo* assays, with complementary endpoints among the assays, are included in the Tier I Screening Battery.

**Question 6**. Are the spreadsheets for data analysis completed and available as indicated in the guidelines?

**Answer 6**. No, unfortunately they are not currently available.

## IV. CONCLUSION

In response to the request received from Colleen Toole, Director of Project Management of Cee Tox, the Endocrine Disruptor Review Team (EDRT) has provided responses to several generic technical questions regarding the conduct of *in vitro* EDSP Tier 1 assays.