AGENDA
Federal Insecticide, Fungicide, and Rodenticide Act
Scientific Advisory Panel Meeting
January 29-February 1, 2013

Prioritizing the Universe of Endocrine Disruptor Screening Program (EDSP) Chemicals Using Computational Toxicology Tools

Docket Number: EPA-HQ-OPP-2012-0818 **OPP Docket Tel: 703-305-5805**

Please note that all times are approximate
(See note at the end of the Agenda)

Tuesday, January 29, 2013

9:00 A.M. **Opening of Meeting and Administrative Procedures** – Sharlene Matten, Ph.D., Designated Federal Official, Office of Science Coordination and Policy, EPA

9:05 A.M. **Introduction and Identification of Panel Members** – Daniel Schlenk, Ph.D., Chair, FIFRA Scientific Advisory Panel

9:15 A.M. **Opening Remarks** – Steven Bradbury, Ph.D. Director, Office of Pesticide Programs, EPA

9:25 A.M. **Overview of EDSP21 Work Plan, Universe of Chemicals and Prioritization Scheme** – Mary Manibusan, Director, Exposure Assessment Coordination and Policy Division, Office of Science Coordination and Policy, EPA

10:00 A.M. **Break**

10:15 A.M. **Use of Physico-Chemical Properties to Exclude Chemicals from EDSP Screening" –** Ray Kent, Ph.D., Health Effects Division, Office of Pesticide Programs, EPA

10:45 A.M. **Estrogen Adverse Outcome Pathway and Estrogen Receptor Expert System –** Patricia Schmieder, Ph.D. and Rick Kolanczyk, Mid-Continent Ecology Division, National Health and Environmental Effects Research Laboratory Office of Research and Development, EPA

12:00 P.M. **Lunch**

1:00 P.M. **Performance of High Through Put (HTP) ER Binding and Transcriptional Activation Assay –**
Richard Judson, Ph.D., National Center for Computational Toxicology, Office of Research and Development, EPA

2:30 P.M. **Break**

2:45 P.M. **Preliminary Analysis of *in vitro* data used as ES TrSet and *in vitro* HTP Assay Data–** Michael Hornung, Ph.D. Mid-Continent Ecology Division, National Health and Environmental Effects Research Laboratory, Office of Research and Development, EPA

3:45 P.M. **Category Based Approach and Strategic Testing to Cover Unknowns –** Patricia Schmieder, Ph.D. and Rick Kolanczyk, Mid-Continent Ecology Division, National Health and Environmental Effects Research Laboratory, Office of Research and Development, EPA

5:00 P.M. **Summary –** Vicki Dellarco, Ph.D., Science Advisor, Office of Pesticide Programs, EPA

5:30 P.M. **Adjournment**

Wednesday, January 30, 2013

9:00 A.M. **Opening of Meeting and Administrative Procedures** – Sharlene Matten, Ph.D., Designated Federal Official, Office of Science Coordination and Policy, EPA

9:05 A.M. **Introduction and Identification of Panel Members** – Daniel Schlenk, Ph.D., Chair, FIFRA Scientific Advisory Panel

9:10 A.M. **Follow-up from Previous Day's Meeting** – Mary Manibusan, Director, Exposure Assessment Coordination and Policy Division, Office of Science Coordination and Policy, EPA

9:20 A.M. **Public Comments**

10:30 A.M. **Break**

10:45 A.M. **Public Comments (continued)**

12:00 P.M. **Lunch**

1:00 P.M. **Panel Discussion of Charge Questions**

Question 1. Overall Prioritization Approach (Section 2.2; also see Section 9)

1.1 Please comment on appropriateness of the overall conceptual approach for prioritizing the EDSP chemical inventory (note: subsequent charge questions address issues associated with specific components of the prioritization approach). In your comments, please address:

The extent to which EPA's description of the process transparently captures and describes the key technical steps of the prioritization scheme and whether there are other scientific considerations the agency should incorporate into its EDSP prioritization scheme; the robustness of the scientific support for the overall approach and, in particular, the logical sequence of filters, and whether changes to the approach could set priorities more efficiently and effectively.

Question 2. Physicochemical Properties (Section 2.2.2)

Not every chemical necessarily needs to undergo EDSP screening and testing. Decisions of whether to screen a chemical for endocrine interaction are made in part based on information about a chemical's inherent properties (*e.g.*, pKa values, molecular weight, reactivity/stability, corrosivity, known functional groups, and charged species). EPA may use this information to identify untestable chemicals or those that are unlikely to pose systemic effects (*e.g.*, endocrine perturbation). Application of filters based on physiochemical properties enables EPA to reduce the chemical universe that would need further evaluation (as illustrated in the white paper, from ~10,000 chemicals to ~5000 or less).

2.1 Please comment on the proposed exclusion criteria for each physicochemical property as appropriate for identifying chemicals that are untestable and/or unable to elicit systemic toxicity. Please include a discussion of the extent to which varying the range of exclusion criterion can influence confidence in identifying chemicals that are untestable and/or unable to elicit systemic effects (i.e., those substances that are unlikely to trigger the molecular initiating event). Please include in your comments:

Whether there are additional chemical characteristics that should be considered (e.g., volatility),

Whether there are additional data sources for each parameter identified (e.g., chemaxon for pKa values, EpiSuite for half-life values, etc.), and how to consider multiple data sources in a weight of evidence approach.

Whether the exclusion criteria cut offs and the confidence that application of the exclusion criteria will reasonably identify those substances that are untestable and/or unable to trigger the molecular initiating event of the ER pathway. Also please discuss options for evaluating cases where uncertainty in a chemical's specific parameter range overlaps a p-chem filter cut off. Please consider in your comments the following exclusion criteria:

pKa values <2 or >11.5;

hydrolysis half-life of <40 days and

charged species of 99% or greater at pH 7

2:30 P.M. Break

2:45 P.M. <u>Question 3.</u> ER Expert System Development (Section 3)

The ER ES was originally built based on a training set (TrSet) of chemicals using *in vitro* assays specifically optimized to measure a well defined endpoint as indicated by ER binding and gene activation and to cover the domain of applicability (in the case of ESv1 food use pesticidal inert ingredients and antimicrobial active ingredients). This ES was the subject of a 2009 FIFRA SAP review. Since that review additional work has been done and additional TrSet data have been generated to cover pesticide non-food use inert ingredients *(i.e.*, ESv2). In addition, an analysis has determined that the ESv2 can cover ~70 percent of the EDSP Chemical Universe that includes SDWA chemicals and fragrances.

3.1 Please comment on the approach used to build ESv2 through chemical testing and effect-based category and read across methods to provide a scientifically defensible approach to predict ER binding potential for a larger number of chemical groups, particularly with regard to defining new groups from within the Mixed Organics and Mixed Phenols. Please indicate any considerations unique to ESv2 that indicate the approach used to develop ESv1 (SAP, 2009a) needs to be modified.

3.2 Building from the ESv1 training set, please comment on the level of scientific confidence that a chemical is unlikely to initiate the ER AOP, if the in vitro assay TrSet data shows no activity. Please comment on the extent to which the level of confidence may vary by chemical category.

<u>Question 4.</u> HTP ER Binding and ER Transactivation Data (Sections 4-6)

The agency is proposing to use the HTP data to expand the ER expert system (*i.e.*, ESv3). Therefore, it is important to understand how the HTP data are generated and interpreted for the intended use. As endorsed by the 2010 SAP on *Integrated Approaches to Testing and Assessment Strategy: Use of New Computational and Molecular Tools*, in evaluating computational tools, the agency will consider internationally accepted science principles including the OECD QSAR validation principles of "Clear, Defined Biological Endpoint, Mechanistic Interpretation, Unambiguous Algorithm, Goodness of Fit and

Domain of Applicability." These principles are consistent with and complement the recommendations of the Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC) and considered flexible to be broadly applicable to predictive models or computational tools which may be based on HTP or other *in vitro* data. The principles particularly applicable to this charge question are: a well defined endpoint and mechanistic interpretation. A "well defined endpoint" is intended to ensure clarity in the endpoint data used to build predictive models, chemical categories, or read-across. Any predictions made from the data will inherently contain all uncertainties and limitations in the data measurement and interpretation. The intent of "mechanistic interpretation" is to ensure a mechanistic association between the attributes of a chemical and its interaction with the biological system resulting in the measured endpoint, to the degree possible. The following questions relate to the transparency of the HTP techniques (*e.g.*, how the data are processed and interpreted and the extent of reliability and consistency within an assay and among HTP assays).

4.1 Given the importance of a well defined endpoint to determining if/how endpoint data are appropriately used, the panel is asked to comment on:

Whether the assays are sufficiently described so that others can reconstruct the assay conditions and data analysis and to what extent additional information would be useful?

Whether EPA has described sufficiently the important experimental conditions that affect the assay measurements, and whether EPA has sufficiently discussed their potential impact on assay results and interpretation of the results?

Whether the chemical library (e.g., purity and analysis), chemical exposure (e.g., solvents used, chemical dilutions), and plates and plate layouts provides information necessary for data interpretation?

The adequacy of test concentrations (maximum and minimum concentration tested) and cutoffs, and whether the concentration cutoffs affected some assays results more than others, especially in the context of false negatives.

4.2 With respect to data interpretation, please comment on the approach for defining an active chemical (i.e., to initiate the ER AOP) and an inactive chemical (i.e., unlikely to initiate the ER AOP) compound and whether the method of data interpretation is adequately described and if the rationale for the approach is sufficiently presented. Please include in your comments:

The adequacy of the approach to generate the data plots; how and what parameters are calculated, etc.

How the background data were used to establish the control level,

Whether the process for identifying assay interference is adequately described,

Adequacy of data normalization, outlier identification, curve fitting, background subtractions, and all other data processing and calculation techniques, and appropriateness of the statistical analyses.

4.3 Would you recommend other considerations or approaches to analyzing the HTP data?

5:30 P.M. Adjournment

Thursday, January 31, 2013

9:00 A.M. **Opening of Meeting and Administrative Procedures** – Sharlene Matten, Ph.D., Designated Federal Official, Office of Science Coordination and Policy, EPA

9:05 A.M. **Introduction and Identification of Panel Members** – Daniel Schlenk, Ph.D., Chair, FIFRA Scientific Advisory Panel

9:10 A.M. **Follow-up from Previous Day's Meeting** – Mary Manibusan, Director, Exposure Assessment Coordination and Policy Division, Office of Science Coordination and Policy, EPA

9:20 A.M. **Question 5. Performance Evaluation of the HTP ER Binding and Transcriptional Activation Assays Against a Set of Reference Chemicals (Section 6)**

An important aspect of the Agency's Proposed Chemical Prioritization Approach for the EDSP is to minimize the occurrence of false negatives and to have confidence that all potentially active chemicals (*i.e.,* those that can trigger the MIE) are identified. Therefore, the performance of each of the computational toxicology components of the prioritization process is being evaluated. The performance of each HTP assay has been evaluated using a set of well-documented reference chemicals that represent a diversity of chemical classes and range of potencies. Additional performance metrics have also been evaluated that take into account a number of parameters that are unique to HTP technology.

5.1 Please comment on the selection of reference chemicals and whether they are sufficient to assess the performance of each HTP assay for ER agonists. How well do the reference chemicals represent the range of potencies needed to establish the reliability and relevance of these assays for use in EDSP chemical prioritization for the inert ingredients, fragrances, and SDWA chemicals?

5.2 Please comment on whether the Agency's evaluation of the performance of the HTP ER binding and activation assays has considered and accurately assessed all relevant aspects of the assays. For example, have signal-to-noise ratios, background subtraction and interferences been adequately characterized when describing the strengths and limitations of the assay?

5.3 Based on analyses in sections 5 and 6, please comment on the comparative performance of the 8 HTP assays for detecting ER reference agonists (e.g., do some assays perform differently, and if so to what extent?) Was sufficiently detailed information provided explaining the likely reasons for assay differences, when they are observed (e.g., cutoffs, assay interferences, background differences)?

10:30 A.M. **Break**

10:45 A.M. **Question 6. Analysis of ER Expert Training Set and HTP *in vitro* Assay Data (Section 7)**

To better understand assay responses to a chemical and whether it has the potential to activate the ER-mediated pathway, the agency compared a set of chemicals evaluated with the ER HTP *in vitro* assays with data on the same set of chemicals included as part of the *in vitro* training set data used to build the ER ESv1/2 (ES TrSet).

6.1 Please comment on the agency's assessment of how HTP in vitro assays performed with respect to expectations of performance for chemicals within chemical categories when compared with the in vitro training set data used to build the ESv1/2.

6.2 Please comment on EPA's approach to comparing the ES TrSet data with the HTP data and the interpretations regarding discordant results between the two assay approaches. Please recommend other considerations or approaches to conducting this comparative analysis?

6.3 Given that there may be varying occurrences of false negatives and false positives based on different sources of training set data, please include in your comments options for addressing these uncertainties for different categories in the context of building ESv3?

12:00 P.M. Lunch

1:00 P.M. <u>Question 7</u>. Category-Based Approach and Strategic Testing to Expand the ES applicability domain with additional *in vitro* data (Section 8)

Section 8 describes a category base strategy (as used to build the original ESv1) to expand coverage training set data for the entire EDSP inventory (*i.e.*, the OECD principle that addresses the "domain of application"). The approach described in Section 8 is considered relevant to any *in vitro* assay data that is applicable for measuring a well-defined end point within an AOP under study (in this case, an ER-mediated pathway).

7.1 Please comment on the adequacy and efficiency of the category based approach to select chemicals for testing to expand the training sets in terms of covering the ~1700 chemicals not covered in the domain of ESv2.

7.2 Based on the HTP assay performance (analysis of reference chemicals and comparative analysis with the ES TrSet), please comment on the adequacy of the HTP data for advancing the ER expert system's rules to cover the additional groups of chemicals in ESv3.

7.3 To the extent there are differences between the ES TrSet and HTP data in detecting the ability of chemicals to initiate the ER AOP, especially in the context of minimizing 'false negatives' for low potency compounds, please comment on the strengths and limitations of combining data from the different assays to generate training set data for building the structure based rules within the ESv3. What are the strengths and limitations of using training set data from assays that have the same or similar degree of sensitivity?

2:30 P.M. Break

2:45 P.M. <u>Question 8.</u> In Vitro Testing and Computer Based Simulations: Addressing Active Metabolites (Note: This question is looking toward the future and there is no proposal presented in the white paper. Discussion of the issue of active metabolites in examples are presented in Section 7.4.3)

In the context of building a category rule-based approach, it is important to understand if the biological activity is attributed to the parent compound or a metabolite. It is also important to understand if a metabolite of an 'inactive' parent compound may be able to trigger the ER MEI. The agency would like to take advantage of the growing knowledge of assays systems as well as existing metabolic profiles, computation techniques for predicting metabolites and understanding of ER-binding structure activity relationships to address this area of uncertainty.

8.1 Please provide any initial thoughts on how varying empirical and/or computational techniques could be employed to account for metabolites that may trigger the ER MIE.

Question 9. Use of the AOP and Category-Based Testing Strategies for Other Endocrine Pathways (Section 9)

The agency plans to implement an EDSP chemical prioritization scheme that will use both exposure and effect based metrics. The current paper focused on development of an effect-based approach using the ER pathway and relevant *in vitro* assays. This prioritization methodology draws on several fundamental concepts generally applicable to the development of computational methods. These concepts include understanding inherent chemical properties as well as chemical structure and bioactivity relationships, use of the AOP concept and category-based read across methods to guide the development and application of *in vitro* testing using lower or higher throughput methods and computer-based simulations.

9.1 Please comment on whether the principles and concepts used to develop the ER expert system are generally applicable for any category-based prioritization system for other molecular initiating events (e.g., other AOPs for perturbing estrogen, androgen receptor, and thyroid hormone systems)?

9.2 What lessons have been learned from the development of the ER focused prioritization model that will facilitate more efficient and effective development of an effect-based prioritization model for the androgen pathway.

9.3 What will be the challenges in developing a prioritization using in vitro methods and computer based simulations for the thyroid hormone system?

5:30 P.M. Adjournment

Friday, February 1, 2013 (if needed)

9:00 A.M. **Opening of Meeting and Administrative Procedures** – Sharlene Matten, Ph.D., Designated Federal Official, Office of Science Coordination and Policy, EPA

9:05 A.M. **Introduction and Identification of Panel Members** – Daniel Schlenk, Ph.D., Chair, FIFRA Scientific Advisory Panel

9:10 A.M. **Follow-up from Previous Day's Meeting** – Mary Manibusan, Director, Exposure Assessment Coordination and Policy Division, Office of Science Coordination and Policy, EPA

9:25 A.M. **Discussion of Charge Questions**

10:30 A.M. **Break**

10:45 A.M. **Discussion of Charge Questions (continued)**

12:30 P.M. **Adjournment**

Please be advised that agenda times are approximate; when the discussion for one topic is completed, discussions for the next topic will begin. For further information, please contact the Designated Federal Official for this meeting, Dr. Sharlene Matten, telephone: (202)-564-0130, fax: (202) 564-8382, or email: matten.sharlene@epa.gov.