DRAFT AGENDA

AGENDA
FIFRA SCIENTIFIC ADVISORY PANEL (SAP)
OPEN MEETING
August 25-27, 2009

FIFRA SAP WEB SITE http://www.epa.gov/scipoly/sap/
OPP Docket Telephone: (703) 305-5805
Docket Number: EPA-HQ-OPP-2009-0322

U.S. Environmental Protection Agency
Conference Center - Lobby Level
One Potomac Yard (South Bldg.)
2777 S. Crystal Drive, Arlington, VA 22202

Scientific Issues Associated with The Use of Structure Activity Relationships of Estrogen Binding Affinity to Support Prioritization of Pesticide Inert Ingredients and Antimicrobial Pesticides for Screening and Testing

Please note that all times are approximate (see note at end of Agenda).

Tuesday, August 25, 2009

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

9:10 A.M. **Introduction and Identification of Panel Members**
Janice Chambers, Ph.D., FIFRA Scientific Advisory Panel Session Chair

9:20 A.M. **Welcome and Opening Remarks**
Stephen Owens, Assistant Administrator, Office of Prevention, Pesticides, and Toxic Substances, EPA

9:30 A.M. **Welcome and Opening Remarks**
Laura Bailey, Executive Secretary, FIFRA Scientific Advisory Panel, Office of Science Coordination and Policy (OSCP), EPA

9:35 A.M. **Opening Remarks, Goals and Objective of Meeting**
Steven Bradbury, Ph.D., Deputy Director, Office of Pesticide Programs (OPP), EPA

9:40 A.M. **Status of the U.S. Endocrine Disruptor Screening Program**
Gary Timm, Exposure Assessment Coordination and Policy Division, OSCP, EPA

10:00 A.M. **BREAK**

10:15 A.M. **Prioritizing Chemicals for Screening and Assessment: An Application for the Endocrine Disruptor Screening Program**
Steven Bradbury, Ph.D., Deputy Director, OPP

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10:45 A.M. **(Q)SAR/Expert System to Estrogen Binding Affinity**
Patricia Schmieder, Ph.D. and Richard Kolanczyk, Ph.D., Mid-Continent Ecology Division, National Health and Environmental Effects Research Laboratory-Duluth, Office of Research and Development

12:30 P.M. **LUNCH**

1:30 P.M. **Public Comments**

3:00 P.M. **BREAK**

3:15 P.M. **Charge to the Panel: Question 1**

1) Evaluation of the Expert System in the Context of the Organization for Economic Cooperation and Development (OECD) Validation Principles

As discussed in the Preface and Introduction of the white paper, EPA's development of the Quantitative Structure Activity Relationship (QSAR)-based expert system was guided by the OECD principles for (Q)SAR validation. The five principles include demonstration of:

- a well defined endpoint,
- mechanistic interpretation of the model,
- defined domain of model applicability,
- an unambiguous algorithm, and
- appropriate measures of goodness of fit, robustness, and predictivity.

A prototype of the expert system was the subject of an OECD convened peer-consultation in February 2009, at which time the system was evaluated using the (Q)SAR validation principles. Based on input from this peer consultation, the Agency further refined the expert system, particularly as it related to the OECD validation principles. The components to Charge Question 1 address specific issues concerning the (Q)SAR validation principles and the subject expert system in the context of its use to determine the order in which chemicals (i.e., food use inert ingredients and antimicrobial pesticides) will be subject to Tier 1 screening under EPA's Endocrine Disrupter Screening Program (EDSP) (*i.e.*, for prioritization).

1) A. **<u>Biological Endpoint</u>**

The biological endpoint that is predicted by the expert system is relative binding affinity to the cytosolic rainbow trout estrogen receptor (ER). Based on preliminary studies it was anticipated that food use inert ingredients and antimicrobial pesticides would have low relative binding affinities. In addition, an evaluation of the two respective inventories indicated a wide range of structures and associated physical-chemical properties (*e.g.,* solubility, Kow, etc). Consequently, assay methods used to measure binding affinity to establish the training set were designed to detect low levels of binding affinity (*e.g.,* testing to solubility in binding

assays and cytotoxicity or solubility, as appropriate, in slice assays). Confirmatory binding studies (Ki experiments) and transactivation assays were employed to systematically verify that apparent low affinity binding levels represented competitive displacement and translated to ER-mediated transactivation.

Question A1. Please comment on the methods employed and their adequacy for detecting and measuring ER binding affinity for the compounds in the two chemical inventories of immediate interest.

Question A2. In the context chemical prioritization for Tier 1 screening, please comment on the decision to measure binding affinity up to the maximum concentration based on the solution properties of the chemical, rather than using ligand concentration 'cut-off' values of -4 Log Molar to -3 Log Molar, which have typically been used to conclude a compound does not bind to the ER.

Question A3. Please also comment on how any *in vivo* studies that are available for compounds with low receptor binding affinity could be used to provide a relative binding affinity 'cut-off' value either alone or in combination with cut-off values based on the maximum solubility of a ligand in the buffer solution.

1) B. Mechanistic Interpretation

Numerous studies have established the alignment of estrogen and other high affinity ligands within the ER binding domain and indicate that a distance of 10.2 to 11A between the two H-bonding sites and stable (non-flexible) ring structure is optimal for binding. These and other studies lead to the assumption that acyclic compounds would not bind to the ER, although a systematic analysis across a diversity of structures was not available in the literature. In the current study 25 acyclic compounds across 10 classes present in the inventories were evaluated in the training set and none were found to bind at a Relative Binding Affinity (RBA) detection limit of 0.00001%.

Question B1. Please comment on the adequacy of this training set for supporting the expert system's rule that acyclic compounds do not bind to the ER.

Based on studies by Katzenellenbogen *et al.* (2003 and references therein) a working hypothesis in developing the training set was that compounds in the inventories of interest could bind at one site; *i.e.,* the A site or the B site, based on the presence of a hydrogen bond donor or acceptor substituent. The development of the training sets and the resultant ER expert system use a chemical hierarchy based on different

binding mechanisms (*i.e.,* A-B binding sites, A binding site only, B binding site only).

> **Question B2.** Please comment on strengths and limitations of this mechanistic interpretation for selecting chemicals in the training sets and for interpreting the observed binding data.

While ER binding can be an initial step in a toxicity pathway leading to adverse reproductive outcomes (see Figure 1 in the white paper), the ER binding data in the training set, and the associated expert system, were not designed to predict *in vivo* responses. Rather the expert system was designed to predict relative ER binding affinity from a chemical's structure to support the prioritization of food use inert ingredients and antimicrobial pesticides for *in vitro* and *in vivo* Tier 1 screening, which is designed to ascertain if a compound has the potential to interact with the estrogen system.

> **Question B3.** Please comment on the clarity of the white paper in describing the differences in (Q)SAR development when the goal is to predict *in vitro* ER receptor binding from chemical structure vs. when the goal is to predict *in vivo* reproductive/developmental responses from chemical structure. Please indicate if additional discussion in the white paper is needed to establish the relevance of ER binding affinity (either measured or predicted) to interpret the potential for *in vivo* outcomes.

5:30 P.M. ADJOURN

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Wednesday, August 26, 2009

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**
Janice Chambers, Ph.D., FIFRA Scientific Advisory Panel Session Chair

9:15 A.M. **Follow-up from Previous Day's Discussion**
Steven Bradbury, Ph.D., Deputy Director, OPP, EPA

9:30 A.M. **Charge to the Panel: Question 1 (continued)**

1) C. **<u>Model Domain</u>**

The domain of the current ER expert system includes rules to support predictions of ER binding affinity for chemicals in the food use inert ingredient and antimicrobial pesticide inventories.

Question. Please comment on the adequacy of the approach that was used to select chemicals for the training sets in terms of these two inventories.

1) D. Algorithm

The ER expert system provides predictions for each chemical, with each individual prediction traceable to chemical subgroups, binding mechanism and endpoint databases. In developing the expert system several chemical subgroups were identified as chemicals that contain multiple functional groups.

Question D1. Please make suggestions for improvements in presenting the expert rules and their underlying rationale, especially with regard to groups with multiple functional groups.

Question D2. Please also comment on the ability of the expert rules to identify chemicals outside the model domain.

10:15 A.M. BREAK

10:30 P.M. Charge to the Panel: Question 1 (continued)

1) E. Goodness of Fit, Robustness, and Predictivity

Consistent with suggestions by the EDSTAC (1998), and typical processes for (Q)SAR development, the expert system rules were established through an iterative process of defining subgroups, gathering empirical data to refine subgroups rules, followed by collection of additional empirical data to cover the structural domain and/or until a consistent pattern of structural rules and activity emerged. The expert rules permit each chemical to be assigned to subgroups and an associated estimated binding affinity value, accompanied by an explanation of the basis for the estimate as well as of how the estimate compares to measured data for other members of the same subgroup. The 2009 OECD expert consultation report on the expert system recognized that standard statistical methods such as those used to assess regression model QSARs are not necessarily applicable to expert systems whereas transparency and usefulness as described in the white paper are more appropriate parameters for assessing the validity of an expert system. The peer consultation report found the current approach, with individual predictions traceable to chemical subgroups, binding mechanism and endpoint databases, to be appropriate although the report noted that if additional information could be made available it would facilitate future peer-review on this issue.

Question E1. Please comment on the adequacy of information presented in the white paper to evaluate the scientific rationale of how a chemical is processed through the decision logic; i.e., how a chemical is assigned to a subgroup with an associated binding

affinity value; the mechanistic rationale for estimates of binding affinity data, including data for related chemicals; and how it is determined that a chemical is outside of the domain of the expert system.

Question E2. While to date the Agency is not aware of statistical approaches that would provide the means to assess goodness-of-fit or predictivity of expert systems such as the one described here, is the SAP aware of any statistical approaches or data presentations that could be amenable for such evaluations?

1)F. <u>Transparency and Clarity of the Expert System</u>

In its validation principles, OECD recognized the importance of a transparent validation process for the development of (Q)SAR models in order to further enhance their regulatory acceptance of (Q)SAR models.

Question F1. Please provide any additional comment on how well the white paper's summary of the expert system conforms to the OECD validation principles and provide suggestions, as appropriate, to enhance the clarity or transparency of the expert system's development and intended use with regard to the validation principles.

The white paper and associated presentations at the SAP meeting form the basis of the documentation of the expert system.

Question F2. Please provide any suggestions for preparing the system documentation that will enhance clarity and understanding for users.

12:00 P.M. LUNCH

1:00 P.M. Charge to the Panel: Question 2

2) Acyclic Compounds

Acyclic compounds comprise ~58% of the food use inert and antimicrobial inventories. As discussed in Question 1c, acyclic compounds were found to not bind to the ER. Generally, the absence of hydrogen bonding groups, or inappropriate geometry can explain the failure of these chemicals to bind to ER (e.g., see Katzenellenbogen *et al.,* 2003). Prior to the EPA research described in the SAP review, a diverse set of acyclic structures had not been evaluated for ER binding affinity.

Question 2A. Please comment on the extent to which the finding with acyclic compounds in the FI and AM inventories may be broadly applicable to other acyclic compounds. Suggestions on an approach to empirically and efficiently assess a hypothesis that acyclic compounds will not bind to the ER in other chemical inventories would be welcomed.

Question 2B. Please comment on the extent to which the finding with acyclic compounds in the FI and AM inventories can be applied to other nuclear steroid receptors in general. Suggestions on an approach to empirically and efficiently assess a hypothesis that acyclic compounds will not bind to the androgen receptor would be welcomed.

2:00 P.M. Charge to the Panel: Question 3

3) Prioritization for EDSP Tier 1 Screening

OECD member countries have long recognized the potential of (Q)SAR for initial assessments for thousands of untested chemicals and to establish priorities for follow up actions. The OECD "Integrated Approaches to Testing and Assessment" framework has encourage the use of existing knowledge including (Q)SAR to effectively assess and manage large chemical inventories (http://www.oecd.org/dataoecd/45/52/40705314.pdf). In its final report, the EDSTAC (US EPA 1998a) recommended a tiered approach for detecting chemicals with endocrine disrupting potential using a resource-efficient manner that is similar to OECD's Endocrine Disruptor Testing and Assessment Framework (http://www.oecd.org/document/58/0,3343,en_2649_34377_2348794_1_1_1_1,00.html). Like the OECD approach, the framework proposed by the EDSTAC includes use of (Q)SARs and high through put screening assays.

Question. Based on the characteristics of the (Q)SAR-based expert system presented in the white paper, please comment on the Agency's view that the expert system could be employed to support "sorting and prioritizing" food use inert ingredients and antimicrobial pesticides for EDSP Tier 1 screening

3:00 P.M. BREAK

3:15 P.M. Charge to the Panel: Question 4

4) Cross Species Applicability

As discussed in the white paper, when comparable assay systems are used (*e.g.,* comparing recombinant receptors to recombinant receptors; comparing cytosolic receptors to cytosolic receptors; comparing assays with similar total protein concentrations and thus chemical availability) there is general agreement

in measurements of binding affinity across species (fish, rat, human). Thus, the type of assay used appears to explain differences more than species origin of the receptors. To evaluate the applicability of the current expert system based on a trout ER training set for predicting relative binding affinity to human ERα, binding assays using full-length recombinant human ERα and transactivation assays in human T47D cells are in progress with food use inert ingredients and antimicrobial active ingredients. Chemicals selected for human ER testing are based on predictions from the current expert system and designed to cover each chemical group and bracket the Log Kow ranges within the group. To date results show good species concordance with chemical groups that have members that bind trout ER also having chemicals that bind human ER, although the trend is toward fewer members of a chemical group binding to human ER than to rainbow trout ER (*e.g.,* a more restrictive Log Kow range for binding within a chemical group for human ER). Therefore rainbow trout ER appears to bind more low affinity chemicals within a group but bind the same type of chemicals as does human ER.

Question. Given what is reported in the literature and similarities between human and rainbow trout ER binding affinity observed thus far in the research described in the white paper, please comment on the extent to which use of an expert system based primarily on trout ER binding affinity data is a reasonable effects component for prioritizing food use inert ingredients and antimicrobial pesticides for EDSP Tier I screening.

4:15 P.M. Panel Discussion

5:30 P.M. ADJOURN

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Thursday, August 27, 2009

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**
Janice Chambers, Ph.D., FIFRA Scientific Advisory Panel Session Chair

9:10 A.M. **Follow-up from Previous Day's Discussion**
Steven Bradbury, Deputy Director, OPP, EPA

9:30 A.M. **Panel Discussion (continued as needed)**

10:30 A.M. **BREAK**

10:45 A.M. **Panel Discussion (continued as needed)**

12:00 P.M. **ADJOURN**

Note: 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, Sharlene Matten, via telephone: (202) 564-0130; fax: (202) 564-8382; or email: matten.sharlene@epa.gov.