December 1, 2014

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Designated Federal Official
FIFRA Scientific Advisory Panel Staff
USEPA/OSCP (7201M)
Environmental Protection Agency
1200 Pennsylvania Ave., N.W.
Washington, DC 20460–0001

Dear Dr. Jenkins:

RE: FIFRA SAP Meeting, Integrated Bioactivity and Exposure Ranking – A Computational Approach for the Prioritization and Screening of Chemicals in the Endocrine Disruptor Screening Program; Docket ID EPA–HQ–OPP–2014–0614.

These comments are submitted on behalf of People for the Ethical Treatment of Animals, the Physicians Committee for Responsible Medicine, and the Humane Society of the United States – national animal protection and scientific advocacy organizations with a combined constituency of more than 13 million Americans who share the common goal of promoting reliable and relevant regulatory testing methods and strategies that protect human health and the environment while reducing, and ultimately eliminating, the use of animals.

We appreciate the opportunity to comment on the white paper that is the subject of this FIFRA Science Advisory Panel (SAP) meeting, i.e., the Integrated Bioactivity and Exposure Ranking computational approach for use in prioritization and screening of in the Endocrine Disruptor Screening Program (EDSP).

General Comments

Our organizations are very impressed with and appreciative of the amount of work that the EPA has performed in developing and implementing computational and high throughput screening (HTS) tools to predict both chemical activity in the estrogen receptor (ER) and androgen receptor (AR) pathways and environmental exposure. We are excited about the progress that has been made and the strong likelihood that these tools may soon be ready to replace the uterotrophic and Hershberger EDSP Tier 1 *in vivo* assays, as well as the ER and AR receptor binding assays that, while considered to be *in vitro* assays, rely on the collection and use of animal tissues.

There are two important improvements over the current EDSP encapsulated by the proposed approach: the evaluation and presentation of chemical activities as probabilities, and the inclusion of exposure estimates in prioritization (i.e. Integrated Bioactivity and Exposure Ranking (IBER). Such approaches to prioritizing and screening the universe of EDSP chemicals will lead to a better understanding of the potential endocrine activity of the broad spectrum of chemicals in the EDSP chemical universe as well as to a more efficient and more humane way to rapidly identify those substances that have the greatest potential of causing adverse effects in

human and wildlife populations, and ultimately result in the use of far fewer animals in testing under the EDSP.

The ability of HTS screening to replace the mechanistic *in vivo* Tier 1 assays (i.e. the uterotrophic assay) is promising. The EPA should consider additional ways the information could be used to avoid *in vivo* testing. For example, as described in the white paper, the utility of the *in vivo* Tier 1 uterotrophic and Hershberger assays appears to be related to chemical activation and detoxification. Therefore, it may be possible in the near-term to skip Tier 1 altogether and move on to further evaluation if it can be shown that detoxification is unlikely to play a role in high-IBER scoring chemicals. In addition, as the HTS assays improve and expand coverage and the IBER approach evolves, the true utility of other Tier 1 assays must continue to be reassessed.

While we understand that the focus of the EDSP SAP meetings is on the adequacy and appropriateness of the science, there is often an accompanying lack of clarity with regards to policy – in this case a description of exactly how IBER results will be used to inform decision-making for purposes other than prioritization, such as informing additional Tier 1 and Tier 2 testing. Because of the large animal numbers involved, particularly in Tier 2, consideration of how these methodologies will be used to inform future testing decisions is integral to this ongoing discussion. For example, does the EPA intend to require Tier 2 testing for high-ranking IBER chemicals, or would another level of intermediate testing be considered to further exclude chemical substances? Suggestions have been made for more nuanced tiering or integrated strategies, including addition of a "Tier 1.5"[1] or use of a strategy based on the OECD Conceptual Framework,[2] which could provide a basis for applying IBER information, along with Other Scientifically Relevant Information (OSRI) to inform potential further testing. Can the EPA envision situations in which regulatory decisions could be made with IBER and Tier 1 assays, avoiding Tier 2 altogether? We urge the Agency to consider, and share with the public, how screening results from these methodologies could inform a more integrated approach to testing and assessment.

<u>Specific Comments</u>

1. Consideration of receptor-specific activity vs. potential "pseudo-receptor" activity (Table 2.3 and 2.4, discussion on page 37) as well as representation of chemical activity as a probability (e.g. of agonist or antagonist activity) is particularly helpful in providing additional information regarding uncertainty and/or specificity of chemical behavior and provides a clear advantage of the HTS approach vs. conventional testing.

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[1] Juberg, D.R., Borghoff, S.J., Becker, R.A., Casey, W., Hartung, T., Holsapple, M.P., Marty, M.S., Mihaich, E.M., Van Der Kraak, G., Wade, M.G., Willett, C.E., Andersen, M.E., Borgert, C.J., Coady, K.K., Dourson, M.L., Fowle III, J.R., Gray, L.E., Lamb, J.C., Ortego, L.S., Schug, T.T., Toole, C.M., Zorrilla, L.M., Kroner, O.L., Patterson, J., Rinckel, L.A., and Jones, B.R. (2014). Lessons learned, challenges and opportunities: The U.S. Endocrine Disruptor Screening Program. ALTEX 31, 63-78.

[2] Bishop, P.L. and C.E. Willett. (2013). The Use and Acceptance of Other Scientifically Relevant Information (OSRI) in the U.S. Environmental Protection Agency (EPA) Endocrine Disruptor Screening Program. Birth Defects Res B Dev Reprod Toxicol.101(1):3-22; Juberg, D.R., Gehen, S.C., Coady, K.K., LeBaron, M.J., Kramer, V.J., Lu, H., and Marty, M.S. (2013). Chlorpyrifos: Weight of Evidence Evaluation of Potential Interaction with the Estrogen, Androgen, or Thyroid Pathway. Regul. Toxicol. Pharmacol. 66, 249-263.

2. With respect to the ER model's concordance with results of reference chemicals run in the uterotrophic assay (p.50), the false negative and false positive rates of less than 10% are excellent. The two chemicals that were not predicted correctly appear to be activated or de-activated by metabolic processes not available in the HTS assays used. This underscores the need for more work on reliable methods that incorporate metabolism into the testing system. We urge the EPA to continue its investigation in this area and to make it a priority for future work.

3. On page 52, there is mention of a concordant mathematical relationship between *in vitro* AUC values and the level of *in vivo* potency in the uterotrophic assay. We suggest that the EPA further investigate whether this relationship is maintained between the ER pathway model and higher-tier tests in order to facilitate avoidance of Tier 2 testing and move closer to using *in vitro* and *in silico* approaches for risk assessment. The development of metabolism and toxicokinetic models as described later in the paper are an important part of this goal, but lack of such models need not be a barrier to using the ER pathway model for quantitative prediction in appropriate cases.

4. There appears to be a small error on pp. 58-59 with reference to Figure 2.8. From the sequence of figures, the figure number should probably be 2.16.

5. We see real value in the prioritizing of chemicals for further testing by adding the exposure element to the model. On p. 59, 73 out of about 1,800 chemicals were predicted to have ER activity above the AUC cut-off designated for positive estrogen activity. This number was reduced to just six after applying IBER rankings (Fig. 6.5, p.97), highlighting this approach's utility in focusing attention on only those chemicals with the most potential for adverse effects.

6. Understanding that the work on the AR model is not as far along as that for the ER, we feel greater clarification is needed for the statements on p. 75: "The AR pathway analyses are currently underway and at this time, <u>we propose that adequate confidence has been demonstrated only for the first goal (prioritization).</u> Additional resolution of performance of the AR models and potential utility for contributing to the weight of evidence determination of AR bioactivity will likely be improved by adding additional reference chemicals." As no timeframe is given in the white paper for the completion of the AR work, we question why results of the AR approach could not be used in the short term as part of a WOE, particularly if OSRI is available for a particular chemical that is concordant with those results. It is possible the information on AR bioactivity gathered thus far could be used to help inform decisions about List 2 chemicals and we hope it could be utilized for that purpose.

7. We note the value of the current efforts in helping to identify and prioritize what additional exposure and biomonitoring data will be needed for better predictions of human exposure to chemicals (pp. 84-85). This will hopefully assist in directing and focusing monitoring efforts to provide more useful in the future. As the Agency is aware,

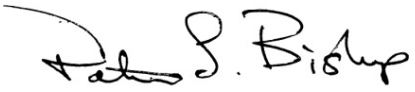
improved biomonitoring is a cornerstone of the 2007 National Academies *Toxicity Testing in the 21st Century* report.

8. With respect to the work being described on the thyroid pathway (p. 99), we acknowledge the EPA as an international leader in attempts to model this pathway and develop useful assays but would also encourage Agency staff to stay engaged with the OECD and global efforts[3] in order to maximize and speed progress in this area and enhance development of the model.

9. We are pleased to see there are plans to model the various other pathways, e.g., steroidogenesis, cell stress, etc., and that the EPA will initiate systematic reviews and curation of the pubertal, AMA, and fish reproduction assays, and look forward to future presentations of these analyses at upcoming SAP meetings.

We are truly excited by the enormous strides the EPA is making in developing and implementing 21st century toxicology and risk assessment methods and the eventual effect this will have on both reducing the use of animals in regulatory testing and increasing the efficiency and accuracy of identifying chemicals of concern.

Sincerely,



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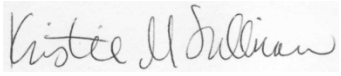

Kristie Sullivan, MPH
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Physicians Committee for Responsible Medicine

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The Humane Society of the United States

[3] Murk AJ, Rijntjes E, Blaauboer BJ, Clewell R, Crofton KM, Dingemans MM, Furlow JD, Kavlock R, Köhrle J, Opitz R, Traas T, Visser TJ, Xia M, Gutleb AC. (2013). Mechanism-based testing strategy using in vitro approaches for identification of thyroid hormone disrupting chemicals. Toxicology in Vitro 27 (2013)1320–1346