Workshop on Lessons Learned, Challenges, and Opportunities: The U.S. Endocrine Disruptor Screening Program RTP, North Carolina, USA - April 23-24, 2013

Summary Report

Background

Over 240 scientists from government, industry, academia, and non-profits participated in a workshop on the Endocrine Disruptor Screening Program (EDSP) in April 2013.[1] The workshop focused on the science and experience to date with the EDSP and identified opportunities to inform ongoing and future efforts to evaluate the endocrine disruption potential of chemicals. The workshop participants discussed the challenges and lessons learned from conducting the EDSP Tier 1 assays in a regulatory context. Suggestions for immediate improvements to Tier 1 screening and for future testing practices within the EDSP were discussed.

In response to public and scientific concern that various environmental chemicals may interfere with endocrine function in humans and wildlife, the U.S. Congress passed amendments to the 1996 Food Quality Protection Act and the 1996 Safe Drinking Water Act requiring the U.S. Environmental Protection Agency (EPA) to implement a screening program to investigate the potential of chemicals to adversely affect endocrine pathways for pesticide chemicals and drinking water contaminants. Consequently, the EPA launched the EDSP and invested considerable time and effort over a number of years to develop and validate the necessary estrogen, androgen, and thyroid (EAT) pathway screening tests and to produce standardized and harmonized test guidelines for regulatory application.[2] In 2009, the EPA issued the first set of testing orders for EDSP screening of 67 pesticides. A total of 50 pesticide actives and 2 inert ingredients were recently screened using the battery of EDSP Tier 1 screening assays (i.e., five *in vitro* assays and six *in vivo* assays) and the data submitted to the EPA for review.[3]

The workshop included presentations from invited speakers across three sessions each followed by panel/audience participation with additional invited experts serving as discussants. The workshop concluded with a fourth open panel/audience session to provide participants a final opportunity to present their perspectives or concerns regarding the future of the EDSP program. This workshop was organized by a committee of volunteers from research institutions, government, industry, academia, and animal welfare and non-profit organizations. Final speaker presentations and more information about the workshop are available at http://tera.org/peer/edsp/ . A workshop report, expanding upon this summary report, will be submitted for publication in ALTEX later this year.

Highlights from Workshop Presentations and Discussions

Session 1: Performance of the EDSP Tier 1 Screening Assays for Estrogen, Androgen, and Thyroid Pathways

- This session focused on the conduct and performance of the eleven Tier 1 assays, highlighting challenges and solutions. Three experts from laboratories involved in the conduct of these assays presented their experiences and discussed their ideas for improvements to future EDSP testing: Dr.

[1] More than 140 scientists representing government, industry, academia, and non-profits participated in person and over 100 additional scientists participated remotely via webinar.
[2] http://www.epa.gov/endo/pubs/edspoverview/index.htm
[3] http://www.epa.gov/endo/pubs/edsp_orders_status.pdf

Colleen Toole (Ceetox), Dr. Leah Zorrilla (ILS, Inc.), and Dr. Katherine Coady (Dow Chemical Company).
  - Several challenges in conducting the assays as prescribed in the EPA test guidelines were highlighted, and technical modifications to the five *in vitro* Tier 1 assays (e.g., Steroidogenesis, Aromatase, Estrogen Receptor [ER], Androgen Receptor [AR], and ER transactivation) were shown to confer significant improvements in performance, including a reduction in potential false positive results. The current design to minimize false negatives makes it difficult to separate true positives from false positives.
  - The Tier 1 *in vivo* mammalian assays provided highly relevant information for determining potential EAT activity through: 1) models which include absorption, distribution, metabolism, and excretion; 2) models which have been optimized to screen for estrogen, androgen, and thyroid modalities, including impacts on the developing endocrine system during pubertal maturation; and 3) the inclusion of redundant endpoints within each assay and across assays (e.g., tissue weights, hormones, histopathology).
  - Few challenges were noted in the conduct of the Hershberger and uterotrophic assays, with the exception that in the Hershberger assay, hepatic enzyme induction, which leads to increased metabolism of exogenous androgen, has the potential to be erroneously interpreted as an anti-androgenic agent. However, dose selection was challenging for all the *in vivo* mammalian assays to ensure that the maximum tolerated dose was achieved, as required in regulatory guidelines. This typically required conducting range finding studies with age-matched animals over a dosing period of 1 to 2 weeks, with an increase in the number of laboratory animals (~ 30 rats) required to obtain an acceptable study to meet EPA's performance requirements.
  - A number of technical challenges were identified during the conduct of the two non-mammalian screening assays (i.e., fish reproductive and amphibian metamorphosis assays) including: 1) ensuring a sufficient fish/tadpole supply and avoiding onset of infection; 2) fecundity performance; 3) selection of the most appropriate test concentrations for each assay; 4) difficulty in meeting performance criteria of <20%CV; and 5) interpretation of assay data (i.e., apical endpoints).
- Workshop participants identified several issues relevant to the conduct, efficiency, and interpretation of EDSP Tier 1 screening assays:
  - Tier 1 assays utilize at least 500 animals for the EDSP Tier 1 battery. Modernized and more effective assays could significantly reduce this number.
  - The EDSP Tier 1 results are intended to be used in conjunction with Tier 2 data (and other relevant scientific information) for risk-based decision-making. However, the endpoints in Tier 1 assays are not designed for risk assessment (i.e., they do not identify adverse effects or dose-response of adverse effects).
  - Test chemical solubility was identified as a significant issue for the *in vitro* and *in vivo* non-mammalian assays.
  - Several technical difficulties were identified for *in vitro* assays including: 1) meeting reference chemical performance, especially for 17α-methyltestosterone (Estrogen Receptor Transactivation assay); 2) laboratory constraints for ER and AR competitive binding assays (e.g., differences in buffers and conducting the assay are challenging for laboratory staff); and 3) meeting the performance criteria for steroidogenesis (e.g., 2-fold Testosterone levels in quality control plate for forskolin induction).
  - The requirement (and value) for testing at such high concentrations (i.e., biological significance) was questioned. The dose levels tested in cell-based assays need to generate data that are both scientifically relevant and meet EPA guidelines.

Session II: Practical Applications of Tier 1 Data

- The second session focused on how to apply relevant information from the current Tier 1 battery to identify potential modes of action and the value of a weight of evidence (WoE) assessment for

evaluating potential interactions with endocrine pathways. The speakers were: Dr. Earl Gray (U.S. EPA), Dr. Sue Marty (Dow Chemical Company), and Dr. Christopher Borgert (Applied Pharmacology and Toxicology, Inc.).

- The value of using a transparent weight-of-evidence approach (WoE) and mode of action assessment to Tier 1 screening data was presented. Using WoE would contribute a robust scientific approach to data evaluation and may provide enhanced confidence in decisions for Tier 2 testing.
- Assays in EDSP Tier 1 were designed to minimize "false negative" results; therefore, using a WoE approach with Tier 1 data would allow for a more scientific approach to data evaluation by examining patterns of effects across assays. This provides some assurance of assay specificity (i.e., some control of the false positive rate) and when appropriate, could provide for a more informed approach to tailor Tier 2 testing.
- A hypothesis-based WoE framework was recently published utilizing data from Tier 1 screening assays.[4] This methodology is undergoing refinement to apply quantitative (relevance) weighting to each endocrine endpoint in the Tier 1 assays, depending on whether an endpoint is a primary indicator of endocrine activity (i.e., specific for an endocrine mode of action [MoA] hypothesis), a secondary indicator of endocrine activity (responsive to the MoA, but not as specific) or supportive data to be used in conjunction with other evidence. Response weighting also is employed, in which test chemical responses are compared to the range of responses elicited by positive and negative control compounds. The approach is designed to be transparent, testable, objectively reproducible, updatable, and biologically plausible.
- A logic-based decision-tree strategy for staging the EDSP Tier 1 screening assays was presented. Specifically, two *in vivo* assays (i.e., fish short-term reproduction and male rat pubertal male assays) were proposed as "Gatekeepers". Using this proposed framework, if both assays yield negative results for potential endocrine activity, then a chemical would be placed in a "HOLD" box and other Tier 1 assays would not be conducted. Conversely, if the "Gatekeeper" assays detected any positive results then additional specific assays would be conducted on a case-by-case basis, depending upon the specific E, A, or T effects observed in the two "Gatekeeper" assays.
- Due to challenges in interpreting Tier 1 data, and concern with the extensive animal use and cost of Tier 2, the potential value of incorporating a possible "Tier 1.5" screening strategy was proposed *in lieu* of moving directly to Tier 2 for chemicals with positive Tier 1 results. Tier 1.5 could be conducted following Tier 1 screening, and could utilize additional or refined *in vitro* or short-term *in vivo* assays to confirm equivocal Tier 1 screening results, or explore potential effects and modes of action in more detail prior to the selection and initiation of extensive Tier 2 testing.

- Workshop participants identified and discussed several important issues regarding the practical application of Tier 1 data:
  - Exposure and dose are two critical issues that should be given increased consideration for future Tier 1 screening programs. If the potential for human and wildlife exposure is minimal a subset of Tier 1 screening might be deemed appropriate (e.g., focus on mammalian assays for potential human exposures, and fish/amphibian assays for wildlife exposures), or additional testing might not be warranted.
  - Incorporation of a critical systematic evaluation of existing Tier 1 data and other relevant scientific information prior to implementation of Tier 2 testing in multiple species was suggested. This type of evaluation could enable the development of a modernized, tailored testing approach for each chemical. This systematic review should be conducted prior to screening EDSP List 2 compounds.
  - Many workshop participants commented positively on the proposed Tier 1.5 screening strategy.

[4] Borgert CJ, Mihaich EM, Ortego LS, Bentley KS, Holmes CM, Levine SL, Becker RA. Hypothesis-driven weight of evidence framework for evaluating data within the U.S. EPA's Endocrine Disruptor Screening Program. Regul Toxicol Pharmacol. 2011 Nov;61(2):185-91.

- The assessment and application of alternative data (e.g., ToxCast, Tox21, adverse outcome pathways) could be highly relevant to replace Tier 1 assays, provided scientific validation of alternative approaches has been achieved for regulatory decision-making.

Session III: Considerations for Future Endocrine Testing

- This session provided perspectives on the future of endocrine screening and the promise of *in vitro* high-throughput analyses, toxicity pathways, and prediction models. The speakers were: Dr. David Dix (U.S. EPA), Dr. Melvin Andersen (The Hamner Institutes for Health Sciences), Dr. Thomas Hartung (Center for Alternatives to Animal Testing, Johns Hopkins), Dr. Catherine Willett (Humane Society of the United States), Dr. Lisa Ortego (Bayer CropScience), and Dr. Thaddeus Schug (NIEHS).
  - In the near future, EPA intends to utilize high throughput screening (HTS) assays and computational modeling (e.g., ToxCast) to prioritize chemicals for the EDSP and in the long-term replace Tier 1 screening. ToxCast chemical screening methods may ultimately replace one or more assays, or perhaps even the entire current EDSP Tier 1 screening battery, increasing speed and efficiency while significantly reducing animal use.
  - A framework approach for a validation strategy that would provide the evidence required for acceptance of HTS assays and prediction models for regulatory applications was outlined. Enhanced communication through peer review publications, greater use of independent science advisory boards, and systematic collaborative reviews would aid in this process.
  - Promising research by the Hamner Institute to design scientifically robust prediction models to elucidate dose-response behaviors at low, environmentally relevant levels of exposures that would be considered sufficient for safety assessments with estrogenic compounds without utilizing whole animal toxicity studies was discussed. The research is based upon estrogenic pathways in a human cell line, and uses molecular probes to elucidate pathway dynamics, cellular perturbations, and reverse toxicokinetics to predict regions of safety for exposures to specific compounds.
  - Overviews of the Pathways of Toxicity research program at Johns Hopkins University and the Evidence-based Toxicology Collaboration, a synergy of U.S. and European stakeholders aimed at developing tools from evidence-based medicine to toxicology were presented.
  - A multi-tiered approach, similar to the Organisation for Economic Co-operation and Development (OECD) Endocrine Disrupter Testing and Assessment (EDTA) Framework, was proposed to improve the current EDSP structure. The proposed system would enable increased opportunities for chemical specific assessment by sequentially evaluating: 1) all existing information (e.g., physiochemical); 2) potential mechanisms of action; 3) tests for potential effects in complex systems or multiple modes of action; and 4) assays measuring adverse outcomes and dose-response. Adapting such a structure would allow the incorporation of new methods and assessment tools as they are developed, facilitating the transition from the current Tier 1 assays to the envisioned fully *in vitro* Tier 1 battery.
- Workshop participants discussed the future of endocrine screening:
  - HTS assays and adverse outcome pathways are envisioned to be applicable to both mammalian and non-mammalian animal models. For example, EPA is funding the development of adverse outcome pathways and endocrine assays in fish and invertebrate models. Similar studies are currently being conducted with a subset of Tox21 chemicals in *C. elegans*.
  - Enhanced predictive tools and stringent validation practices are necessary to achieve future regulatory acceptance of molecular pathways for toxicity testing and hazard risk assessment.
  - Explicit consideration of human and ecological exposure potential would be valuable to aid in prioritization for the EDSP. This would require additional tools for adequately quantifying exposure to evaluate the large numbers of chemicals in EPA's proposed EDSP universe.[5]
  - Current collaborations focused at improving exposure assessment and reverse dosimetry screening in HTS assays show promise for developing a risk-based decision tool. This could initially be used for priority setting and eventually for safety assessment purposes.

[5] http://www.epa.gov/endo/pubs/edsp_chemical_universe_list_11_12.pdf

- Collaboration, coordination, and communication amongst the regulatory community and stakeholders is vital to ensuring the continued progress in development and application of HTS assays and computational profiling prediction models for improving both the EDSP and understanding of a chemical's potential for endocrine disruption.

Session IV: Participant Discussion

- In the final session, workshop participants were provided an extended opportunity to discuss their experiences, perspectives, and concerns regarding the future of the EDSP: A number of common themes emerged from attendees' comments and suggestions during the final session and throughout the workshop:
  - Some participants suggested that a critical review and update of current EPA EDSP Tier 1 testing guidelines, with a focus on improving data quality and/or relevance would be beneficial given the experience gained with the first set of 50 substances. The issue was raised as to whether additional EDSP Tier 1 screening should be put on hold for a year or so while the current battery of tests (and the assessment process for WoE) are evaluated and improved.
  - Additional discussion centered on the proposed two-assay Gatekeeper approach as a possible improvement to staging and conduct of the EDSP Tier 1 battery.
  - A number of participants suggested that consideration of information on human exposure levels (e.g., biomonitoring) would enable better-informed testing, and suggested that chemicals with lower margins of exposure could be addressed first.
  - A guideline detailing a definitive approach to dose setting was proposed; this would be particularly useful for the problematic Tier 1 assays.
  - A robust WoE approach could align available Tier 1 data with potency and exposure information to better inform decisions on what, if any, specific additional Tier 2 testing would be warranted.
  - Several participants spoke favorably about the proposed logic-based decision-tree strategy that would tailor EDSP Tier 2 testing.
  - The development, validation, and implementation of modernized alternative methods that reduce, refine, and replace the use of animals should be a goal of the screening and testing program.
  - Improved venues for stakeholder engagement on EDSP HTS approaches with the EPA would be very useful in advancing the development and application of such methods into the EDSP.
- Interest in enhanced EPA and stakeholder engagement in the OECD activities on Adverse Outcome Pathways (AOP) and Integrated Approach to Testing and Assessment (IATA) as a possible means to transition from conventional testing to Tox21 based approaches over time, as the science evolves and as experience is gained.

For more information about the workshop, including speaker abstracts and presentations, visit: http://tera.org/peer/edsp /