ENDOCRINE SOCIETY
Hormone Science to Health

August 14, 2015

David J. Dix, Ph.D.
Director, Office of Science Coordination and Policy
US Environmental Protection Agency
1200 Pennsylvania Ave. NW
Washington, DC 20460

Re: EPA-HQ-OPPT-2015-0305

Dear Dr. Dix,

The Endocrine Society appreciates the opportunity to comment on the incorporation of high-throughput (HT) assays and computational tools in the Environmental Protection Agency's (EPA) Endocrine Disruptor Screening Program (EDSP). We applaud the EPA for developing a process to rapidly screen thousands of chemicals for the ability to interact with the endocrine system, which is particularly important given the expanding universe of chemicals that have the potential to interfere with hormone action.

Founded in 1916, The Endocrine Society is the world's oldest, largest and most active organization devoted to research on hormones and the clinical practice of endocrinology. The Society's membership consists of more than 18,000 scientists, physicians, educators, nurses and students in 122 countries. Included among our members are the world's leading experts on hormones and the endocrine effects of environmental chemicals. Through published scholarly articles, meetings, and other communications, both in the U.S. and internationally, the Society provides expertise to regulators, policymakers, and other stakeholders on endocrine disrupting chemicals (EDCs). To ensure that the EDSP serves as an effective screening tool, we identify several recommendations for modification as ToxCast becomes more fully integrated into the EDSP.

Ensuring Transparency and Reporting to the Community

The Endocrine Society has been engaged in efforts to promote greater awareness and utilization of ToxCast. Society staff attended the April 2014 joint EPA and FDA workshop on new computational toxicology tools, and the Society sponsored a workshop at the International Congress of Endocrinology and the Endocrine Society's 96th annual meeting on computational toxicology and the EDSP. More recently we participated in the Environmental Defense Fund meeting entitled Elucidating Environmental Dimensions… New Tools from Federal Chemical Testing Programs. Our members report significant confusion about how the ToxCast model works, and that an in-depth knowledge of the computational code is required for deriving information about the underlying data. They also expressed frustration that data elements were unstable, sometimes changing overnight without notice. This raises important questions about the reproducibility of the model and its ability to reliably identify chemicals over multiple iterations. Transparency would be facilitated through better reporting requirements (e.g., information on stock chemicals used, whether assays were run in

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duplicate or triplicate) and a description of how the model treats inconclusive data, specifically whether further testing would then be performed on Tier 2 assays.

Hormones act at very low concentrations, and biologically meaningful responses may be elicited at equally low concentrations of exogenous chemicals. This can manifest as a non-monotonic dose response (NMDR). It is not clear how the ToxCast model treats NMDR, we recommend that this treatment be made explicit. As we state in our response to EPA's draft paper "State of the Science on Nonmonotonic Dose Response", "reliance on the assumption of monotonic – if not linear – dose response, … represent significant departures from what modern science tells us."

Ensuring Accuracy, Reproducibility, and Comprehensiveness

While the Endocrine Society appreciates that the proposed ToxCast HT assays for estrogenicity will provide more coverage than the existing binding assays, a fundamental problem is that results are highly variable across the ToxCast assays. It is also unclear how different the ToxCast assays perform relative to the original binding, transcriptional activation, and uterotrophic assays of the Tier 1 battery. For instance it will be critical to screen chemicals for both estrogenicity and anti-estrogenicity. We note that ToxCast assays have thus far identified 0 chemicals of concern out of the first 52 pesticide active and inert chemicals tested. However, the original Tier 1 battery identified 32 chemicals that showed potential interaction(s) with estrogenic, androgenic or thyroidogenic pathways from that initial 52 chemicals tested – 14 of which were deemed "not of concern" and 18 determined to be of possible concern. Of those 18 chemicals of concern, 14 were potentially estrogenic. This discrepancy would be expected to significantly impact which chemicals would then pass to Tier 2 testing. Although the pubertal, aromatase, and fish short term reproduction assays remain a requirement of testing for estrogenic activity, even with the new ToxCast assay replacements, it is not clear if these three assays can account for this difference in detection of estrogen activity seen between the original Tier 1 battery and the ToxCast replacement. Given the discrepancies between the original Tier 1 battery, and the current ToxCast assays and computational models for the Tier 1 battery, we suggest that EPA clarify how the assays and models perform relative to the original Tier 1 battery. The EPA should also publish a clear plan on how discrepancies will be addressed. The Society feels strongly that the replacement assays must be a demonstrable improvement over existing assays in terms of coverage and detection of potential estrogenic activity.

Furthermore the Endocrine Society is concerned, given the unknown differences in performance between the original Tier 1 assays and the replacement ToxCast assays/model for estrogenicity, that registrants testing chemicals could perform both the ToxCast assays/model and the original Tier 1 assays and elect to submit only the data that detect fewer "hits" for estrogenic activity, thus avoiding further testing. A better understanding of how the two assay batteries compare is important for this reason, as is a clearer description to the public of how the data will be acquired and used (i.e., if the chemical companies test a chemical under the ToxCast battery of assays are they required to submit the data and vice versa).

We note that there are many potential mechanisms for estrogen action other than nuclear-receptor mediated responses, such as epigenetic effects and nongenomic signaling through "nuclear" receptors in other cellular locations. Furthermore, different tissues may have different sensitivities to estrogenic chemicals at different ages and developmental stages, and important information regarding the processing of chemicals during liver metabolism will be missed in ToxCast unless chemicals first are run through liver microsomes. We are concerned that the endpoints used for estrogenicity in EDSP Tier 1 screening are not the most sensitive endpoints; for example, the brain and uterus have widely different sensitivities to BPA that may be altered by age and reproductive status.[1] We therefore recommend that EPA explore the relationship between ToxCast screens for estrogenicity and non-guideline assays that capture both metabolism and more sensitive endpoints.

The Endocrine Society is principally concerned that EPA anticipates expanded utilization of HT assays for the screening of bioactivity in other pathways, such as androgen and thyroid pathways. We know that these systems present significant challenges that will require a deep scientific understanding of the relationship between the assay battery and the ability to predict toxicity. We do not consider the performance of the existing ToxCast assays for androgen and thyroid interference ready for broader application, and we urge EPA to work with endocrinologists and all relevant stakeholders to identify appropriate HT assays that perform comparably or improve upon existing standards.

Ongoing Process Improvement

The Endocrine Society is encouraged by EPA's efforts and partnerships to engage the broader scientific community in ToxCast implementation plans. The EPA should continue to reach out to stakeholders on a regular basis to ensure that assays are monitored and curated via periodic updating and/or replacement. A parallel process to re-evaluate chemicals regardless of prior screening results must be implemented to ensure that compounds can be interrogated by updated systems, thereby minimizing false negatives. As ToxCast performance improves, we encourage EPA to begin evaluating mixtures of chemicals for synergistic effects in accordance with the EDSP Comprehensive Management Plan[2]. This will more accurately mimic real life situations where people are exposed to multiple chemicals simultaneously.

We anticipate that deep stakeholder involvement will enable EPA to overcome the current limitations of HT screening for other hormone pathways, including androgen and thyroid. We maintain that the active involvement of endocrinologists and endocrine scientists, defined as individuals actively contributing new knowledge to the field of endocrinology, will be absolutely necessary as EPA continues to expand the utilization of HT assays in the EDSP.

[1] Rebouli, M.E., et al. (2014). Investigation of the Effects of Subchronic Low Dose Oral Exposure to Bisphenol A (BPA) and Ethinyl Estradiol (EE) on Estrogen Receptor Expression in the Juvenile and Adult Female Rat HypothalamusToxicol Sci. 140, 190–203.
[2] http://www.epa.gov/endo/pubs/EDSP_Comprehesive_Management%20Plan_%20021414_f.pdf Accessed August 4, 2015.

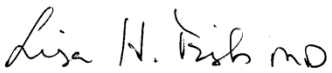
Conclusion and Recommendations

In summary, we urge EPA to consider the following recommendations to improve the EDSP through the integration of HT assays. Specifically, EPA should:

- Provide detailed assay protocols and instructions for researchers on how to access underlying endocrine data for the ToxCast model.
- Notify the research community about any changes to the underlying data and update the model information on public web sites whenever altered models are used internally.
- Provide more transparent information on
  - How the ToxCast data are evaluated for endocrine effects in general, and
  - How these effects compare to those determined in the original Tier 1 battery
- Describe how the ToxCast model will be able to identify NMDR and nongenomic effects.
- Ensure that ToxCast is able to capture non-ER-mediated estrogenic pathways and consequences of metabolism.
- Work with endocrine scientists and other stakeholders to identify suitable assays prior to implementing HT screening for androgen, thyroid, and other hormone pathways in EDSP.
- Implement processes to periodically incorporate new assays and reevaluate previously screened chemicals.

The Endocrine Society supports and appreciates EPA's efforts to improve the EDSP and minimize risks due to exposures to EDCs. We look forward to further discussions to ensure that EPA is able to expeditiously identify chemicals that may interfere with endocrine systems. Thank you for considering the Endocrine Society's comments. If we can be of any further assistance in your efforts, please do not hesitate to reach out to Dr. Joseph Laakso, Associate Director of Science Policy at jlaakso@endocrine.org.

Sincerely,



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