EDC testing in the future: Exploring roles of pathway-based in silico, in vitro and in vivo methods

Gerald Ankley[1], Daniel Villeneuve[1] and Tom Hutchinson[2]

[1]US Environmental Protection Agency, 6201 Congdon Blvd., Duluth, MN, 55804, USA; [2]Centre for Environment, Fisheries and Aquaculture Science, Barrack Rd., The Nothe, Weymouth, DT4 8UB, UK

Email contact: ankley.gerald@epa.gov

1. Introduction

Many thoroughly validated, robust tests with both mammalian and non-mammalian models have been developed to identify chemicals with the potential to impact endocrine pathways associated with the hypothalamic-pituitary-gonadal (HPG) and –thyroidal axes. In the US, for example, the legislatively-mandated endocrine disruptor screening program (EDSP) employs 10 different Tier 1 screening assays to identify chemicals with the potential to affect endocrine function. Although highly effective in identifying endocrine-disrupting chemicals (EDCs), the full Tier 1 battery of tests is relatively resource-intensive. For example, the battery employs six different *in vivo* assays with rats, fish and amphibians that, in some instances, can last up to 30 days. Given current challenges relative to extensive testing, both in terms of resource availability and animal use, it is unlikely that the extant Tier 1 screening battery realistically could be used to assess the many thousands of chemicals that might be of concern relative to endocrine activity.

Recent years have seen the development of many tools that, theoretically, could provide the basis for more rapid, resource-effective screening and testing of chemicals that affect specific biological pathways. These tools include short-term *in vivo* tests (featuring molecular and biochemical responses collected, for example, via genomic techniques), *in vitro* assays (also focused on molecular/biochemical responses, often in systems amenable to high through-put [HTP] testing), and computational techniques (e.g., QSAR modeling; read-across methods). There are challenges, however, relative to the implementation of these types of tools and associated endpoints in regulatory toxicology. One of the major challenges involves translation/linkage of responses at lower levels of biological organization to alterations in individuals (and, by extension, populations) that are relevant to risk assessment (e.g., decreased survival, growth, reproduction).

2. Materials and Methods

The adverse outcome pathway (AOP) framework recently described by Ankley et al. [1] provides a logical basis for incorporating data from pathway-based *in vivo*, *in vitro* and computational tools into regulatory testing programs. The frameworks provides a means for making explicit linkages between molecular initiating events (e.g., receptor binding, enzyme inhibition), which are the focus of most pathway-based tools, and outcomes meaningful to risk assessors/managers. Recently-initiated efforts through international bodies such as the Organization for Economic Cooperation and Development (OECD) are promoting the AOP framework as a basis for more efficient assessments of chemical risk. Hence, it is timely both from a scientific and regulatory perspective to apply the AOP framework to the challenge of EDC screening and testing.

3. Results and Discussion

A number of AOPs relevant to EDC screening/testing already have been developed for effects of chemicals on fish reproduction. An illustrative example will be presented relative to the impacts of chemicals that depress vitellogenin (VTG) synthesis in female fish. Specifically, inhibition of the steriodogenic enzyme aromatase by some pesticides or pharmaceuticals (a molecular initiating event) decrease plasma 17β-estradiol (E2) concentrations, thereby decreasing activation of the estrogen receptor and the associated hepatic production of VTG [1]. The depression in VTG production results in reduced ovarian deposition of the egg yolk protein (which can be visualized histologically), leading to decreased egg production and, ultimately, decreased populations of fish (adverse outcome). This type of knowledge provides a clear basis for translating effects such as *in vitro* inhibition of aromatase or short-term *in vivo* changes in plasma E2 or VTG concentrations into responses useful for risk assessments (decreased fecundity).

The AOP framework also has been applied successfully to other molecular initiating events within the HPG axis that control reproduction and development in fish, including activation of the estrogen and androgen receptors, antagonism of the androgen receptor and inhibition of a number of enzymes involved in steroid synthesis other than aromatase [2]. This presentation will explore progress made relative to these pathways, and provide concrete examples of how this knowledge is (a) supporting development of short-term, pathway-based *in vivo* methods for screening EDCs with small fish models (e.g., fathead minnow, zebrafish), and (b) enhancing interpretation of *in vitro* HTP data.

4. References

[1] Ankley, G.T., R.S. Bennett, R.J. Erickson, D.J. Hoff, M.W. Hornung, R.D. Johnson, D.R. Mount, J.W. Nichols, C.L. Russom, P.K. Schmieder, J.A. Serrano, J. E. Tietge and D.L. Villeneuve. 2010. Adverse outcome pathways: A conceptual framework to support ecotoxicology research and risk assessment. Environ. Toxicol. Chem. 29, 730-741.

[2] Ankley, G.T., D. Bencic, M. Breen, T.W. Collette, R. Conolly, N.D. Denslow, S. Edwards, D.R. Ekman, K.M. Jensen, J Lazrochak, D. Martinovic, D.H. Miller, E.J. Perkins, E.F. Orlando, N. Garcia-Reyero, D. L. Villeneuve, R.-L. Wang and K. Watanabe. 2009. Endocrine disrupting chemicals in fish: Developing exposure indicators and predictive models of effects based on mechanisms of action. Aquat. Toxicol. 92, 168-178.

Acknowledgement: This abstract does not necessarily reflect the views of the US Environmental Protection Agency.