The Endocrine Disruptor Screening Program: Tier 1 Screens and Tier 2 Tests

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ABSTRACT

EPA's Endocrine Disruptor Screening Program (EDSP) was initiated in 2009 -2010 with the issuance of test orders requiring manufacturers and registrants of 57 pesticide active ingredients and 9 pesticide inert/high production volume chemicals to evaluate the potential for these chemicals to interact with the estrogen, androgen and thyroid hormone systems. The EPA Tier 1 endocrine screening battery (ESB) consists of 11 distinct assays comprising both *in vitro* and *in vivo* test systems. Much effort has gone into developing and standardizing these screens. However, there are still challenges in utilizing the results to identify a substance's potential to interact with the endocrine system of humans and wildlife as some of the ESB methods lack specificity for differentiating potential endocrine-mediated responses from responses via other modes of action or via general toxicity. In addition, screening of compounds using the ESB is not a trivial undertaking as the ESB can take many years to complete and costs $750,000 to $1,000,000 per chemical. The Tier 1 testing battery is intended to be evaluated in its entirety in a weight-of–evidence approach to determine whether or not a test chemical potentially interacts with the endocrine system. If results from the Tier 1 battery are considered indicative of a potential interaction, then definitive dose-response testing would likely be done in Tier 2 to further identify the potential hazard and characterize risk.

Tier 1 Screening

- To identify the potential of chemicals to interact with the estrogen, androgen and thyroid hormone systems
  - Tier I is not intended for risk assessment; prioritization for further testing
- Five *in vitro* and 6 *in vivo* assays
- 890 series guidelines, some with comparable OECD guidelines
- Maximize sensitivity to minimize false negatives
- Some endpoints are apical in nature, making it difficult to distinguish between endocrine and non-endocrine responses
- Battery approach with deliberate redundancy
  - The fact that a substance may interact with a hormone system does not mean that when it is used it will cause adverse effects in humans or ecological systems.

Cost $750,000 to $1,000,000 per chemical

Critical Elements in a Weight of Evidence Evaluation

- Reliability of information
  - Quality of the study, transparency of reporting
- Relevance of the information
  - Appropriate for the question being asked
- Adequacy (or usefulness) of the information
  - Fit for regulatory decision-making
- Consistent pattern of response
  - In support of a particular hypothesis

Recommend an Hypothesis-driven WoE framework
- Borgert et al. 2011 Reg Tox Pharm 61:185-191
- Borgert et al. 2014 Birth Defects Res Part B

Tier 1 Screens

Tier 1 Screen	Guideline
Estrogen Receptor Binding	890.1250
Estrogen Receptor Transactivation	890.1300/OECD 455
Androgen Receptor Binding	890.1150
Aromatase	890.1200
Steroidogenesis	890.1550/OECD 456
Uterotrophic	890.1600/OECD 440
Hershberger	890.1400/OECD 441
Male Pubertal	890.1500
Female Pubertal	890.1450
Fish Short-Term Reproduction	890.1350/OECD 229
Amphibian Metamorphosis	890.1100/OECD 231

Redundancy in Tier 1 Screens

Current EDSP Screen	Mode of Action Covered by Screen							
	E	Anti-E	A	Anti-A	T	E	HPG	HPT
Fish Short-Term Reproduction	x	x	x	(x)	x	x	x	
Amphibian Metamorphosis								x
ER Binding or Transactivation	x	x						
AR Binding			x	x				
Steroidogenesis					x	x		
Aromatase						x		
Uterotrophic	x	x						
Hershberger			x	x				
Pubertal Male Rat			x	x	x		x	x
Pubertal Female Rat	x	x				x	x	x

HPG – Hypothalamic-Pituitary-Gonadal Axis
HPT – Hypothalamic-Pituitary-Thyroid Axis

Tier 2 Quandary

- Tier 2 is for determining adverse effects and to provide data for risk assessment
- In regulatory toxicology we test at levels to achieve some effect
- How will we determine if that effect is specifically endocrine related?
  - Not as much of a problem in the US where regulations are based on risk but,
  - In Europe, deciding if the effect is "endocrine" or not has implications for authorization based solely on hazard
- Weight of evidence and hypothesis testing approach needed

Tier 2 Testing

- To identify and characterize adverse effects on reproductive function and development
- Chronic and multigeneration studies in a range of species
  - Rat 2-generation or extended 1-generation reproduction test
  - Medaka extended 1-generation reproduction test (MEOGRT)
  - Larval amphibian growth and development test (LAGDA)
  - Japanese quail 2-generation toxicity test (JQTT)
- Tier 2 is not considered a battery
  - Specific tests will be selected based on information needed for risk assessment
  - Clarifying studies may be requested to obtain targeted information
- Establish a dose-response relationship for adverse effects
- Provide NOEL/LOEL and other information for risk assessment

Summary

- Tier 1 screens are designed to identify the potential of chemicals to interact with the estrogen, androgen and thyroid hormone systems
  - Tier 1 is designed to be evaluated as a battery
  - Weight of evidence procedures must consider reliability, relevance, and consistency of the data and responses
  - Challenges of overt toxicity, apical endpoints, maximizing sensitivity can result in a high false positive rate
- The US EPA has evaluated the List 1 Tier 1 data in a weight of evidence approach with the entire battery and other scientifically relevant data to determine if the chemical has the potential to interact with estrogen, androgen, or thyroid hormone pathways

- Tier 2 tests are for determining adverse effects and to provide data for risk assessment
  - Tier 2 is not a battery
  - Specific tests will be requested based on a weight of evidence evaluation from Tier 1 and other scientifically relevant data

The Future? Use of ToxCast and Other HTP Methods

- EDSP 21 and the EPA Comprehensive Management Plan target moving towards high-throughput (HTP), *in vitro* screens
- Three main objectives:
  - Prioritization - The near-term goal (<2 years)
  - Screening (Tier 1)- The intermediate-term goal (2-5 years)
  - Replacement (Tier 1, Tier 2 possible) – The long-term goal (>5 years)
- Relevance and reliability to a particular hypothesis, as well as the ability to link to an adverse effect, must be determined
  - Dose-response and specificity important

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