An Adverse Outcome Pathway (AOP) Framework for Screening for Potential Endocrine Disruption

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1996 Legislative Mandates for EDSP

1996 Federal Food, Drug and Cosmetic Act (FFDCA), Section 408(p)

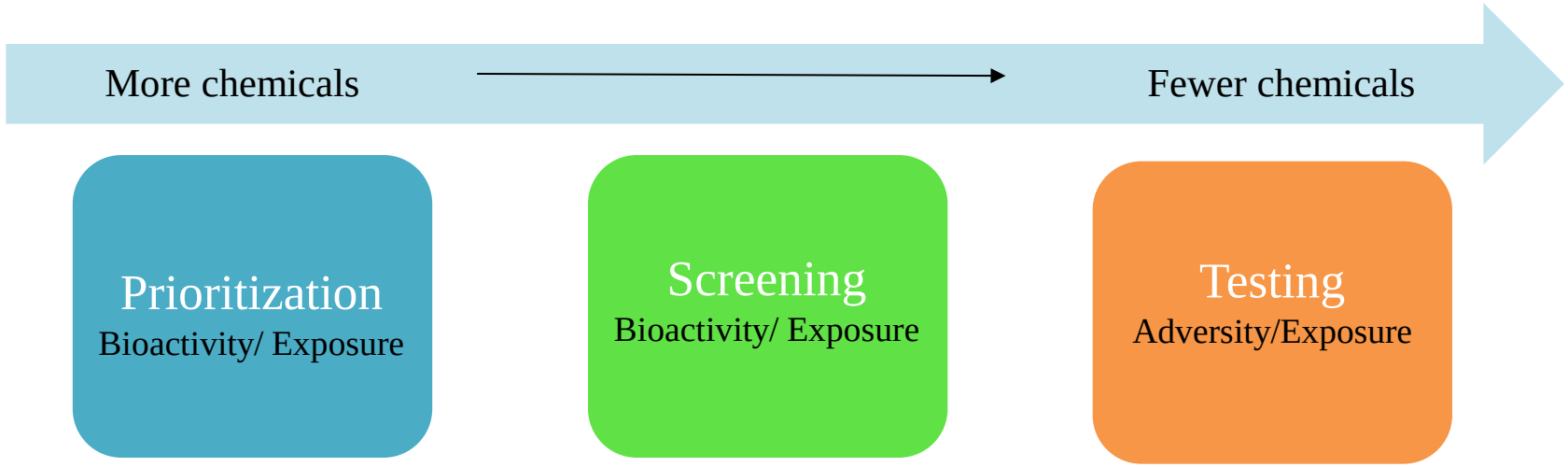
- Requires the U.S. EPA to "develop a screening program using appropriate validated test systems and other scientifically relevant information to determine whether certain substances may have an effect in humans that is similar to an effect produced by a naturally occurring estrogen, or other such endocrine effect as the Administrator may designate."
- In carrying out the screening program:
  - Subsection (3)(A) - "The Administrator shall provide for testing for all pesticide chemicals."
  - Subsection (3)(B) - "The Administrator may provide for the testing of any other substance that may have an effect that is cumulative to an effect of a pesticide chemical if the Administrator determines that a substantial population may be exposed to such substance."

1996 Safe Drinking Water Act (SDWA) Amendments, section 1457

- Provides EPA with discretionary authority to require testing, under the FFDCA Section 408(p) screening program, "of any other substances that may be found in sources of drinking water if the Administrator determines that a substantial population may be exposed to such substance."

1998 Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC)

- Expand protection to include human health and wildlife
- Include estrogen, androgen and thyroid pathways
- Proposed progression:
  - High throughput chemical prioritization based on exposure and bioactivity
  - Tier 1 Screening for bioactivity
  - Tier 2 Testing to determine dose-response and adverse effects



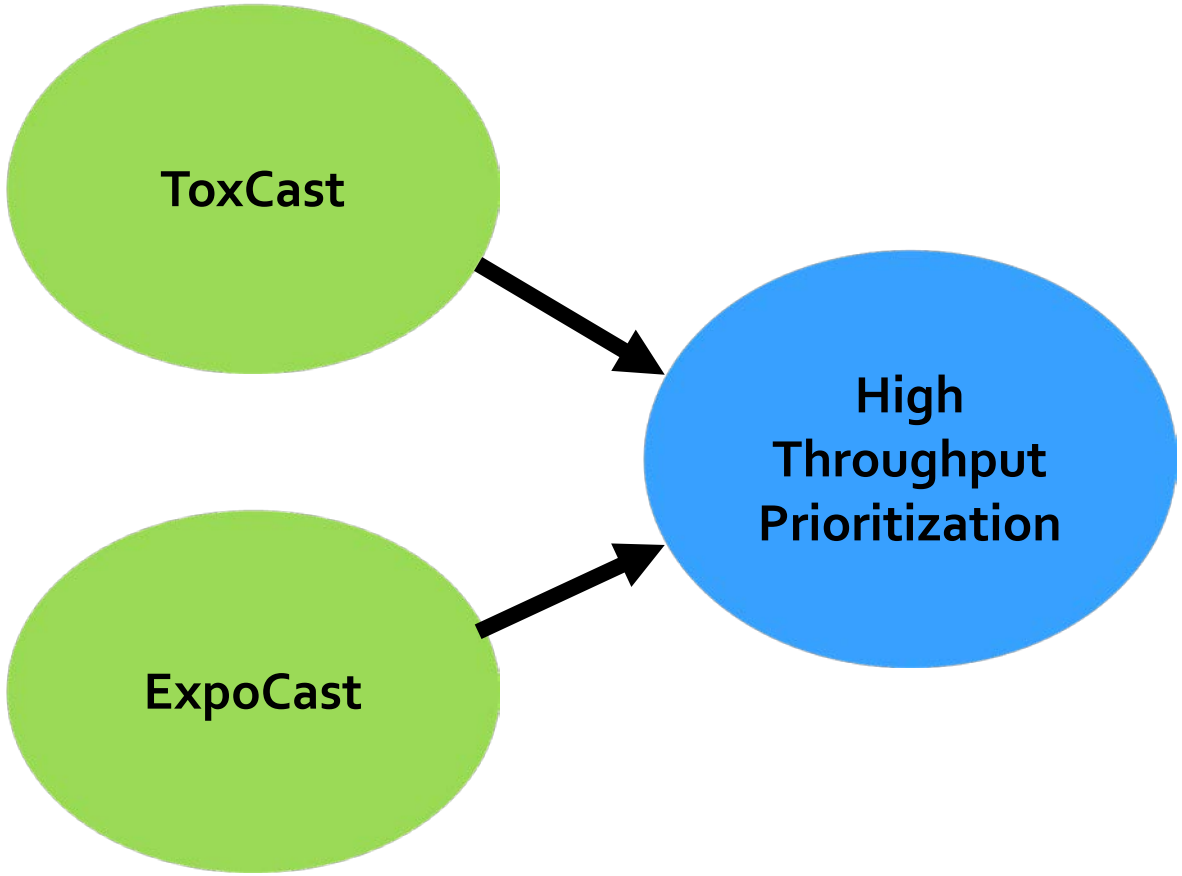
Prioritization

ToxCast

- Expanding use of CompTox (Phys-chem properties, QSARS, etc.) to support screening and prioritization
- Transparent and collaborative

ExpoCast

- Rapid exposure estimation based on readily available chemical use and production data
- Use toxicokinetics to bridge in vitro, concentration-based ToxCast data to in vivo, dose-based Exposures from ExpoCast



Screening

Tier 1

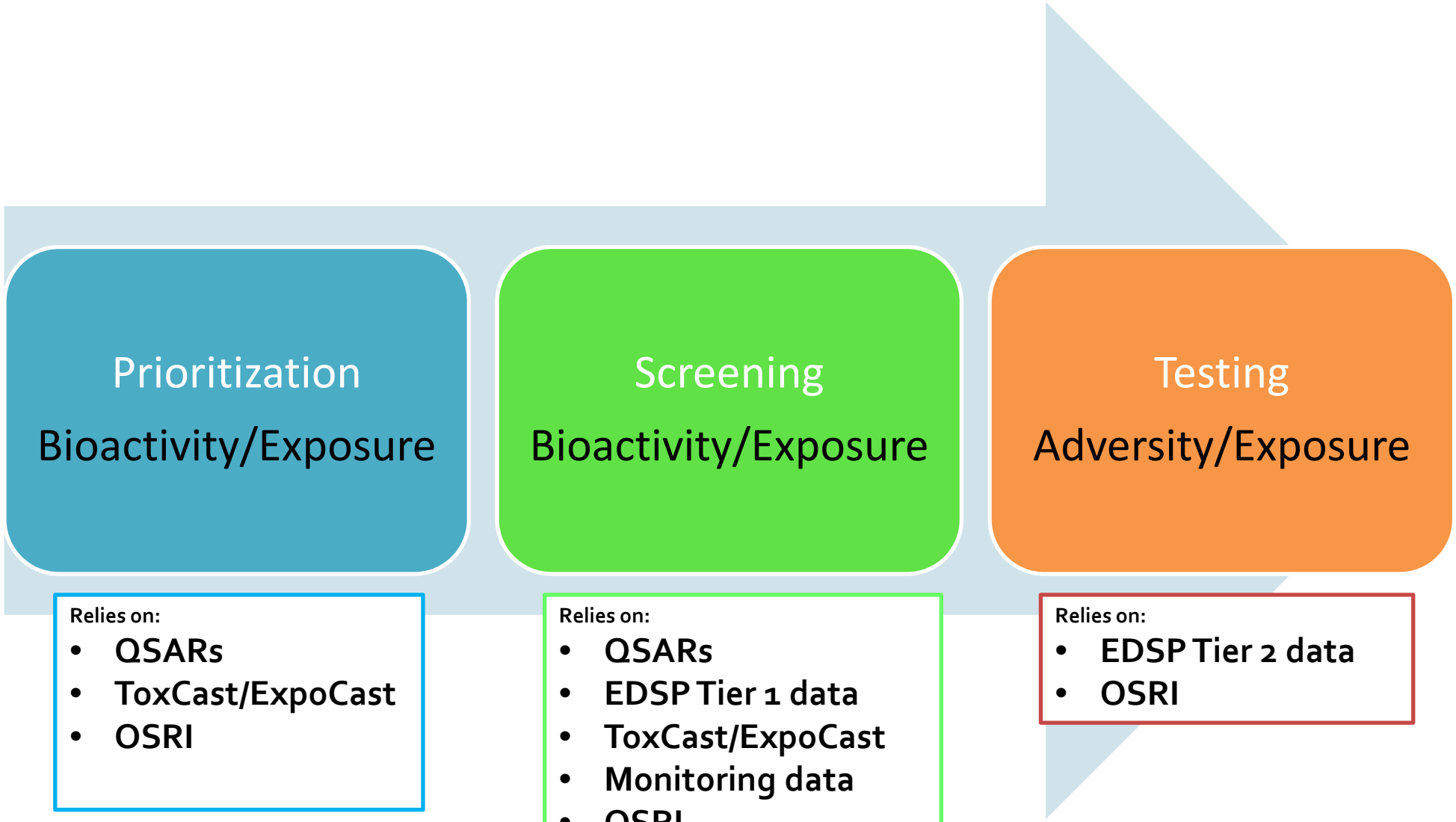
					Steroid Synthesis			
	E	**E-**	**A**	**A-**	**T**	**E**	**HPG**	**HPT**
In vitro								
ER Binding	X	X						
ER Transcriptional Activation	X							
AR Binding			X	X				
Steroidogenesis (H295R)					X	X		
Aromatase (Recombinant)						X		
In vivo								
Uterotrophic	X							
Hershberger			X	X				
Pubertal male			X	X	X		X	X
Pubertal female	X	X				X	X	X
Fish Reproductive Screen	X	X	X	X	X	X	X	
Amphibian Metamorphosis								X

Testing

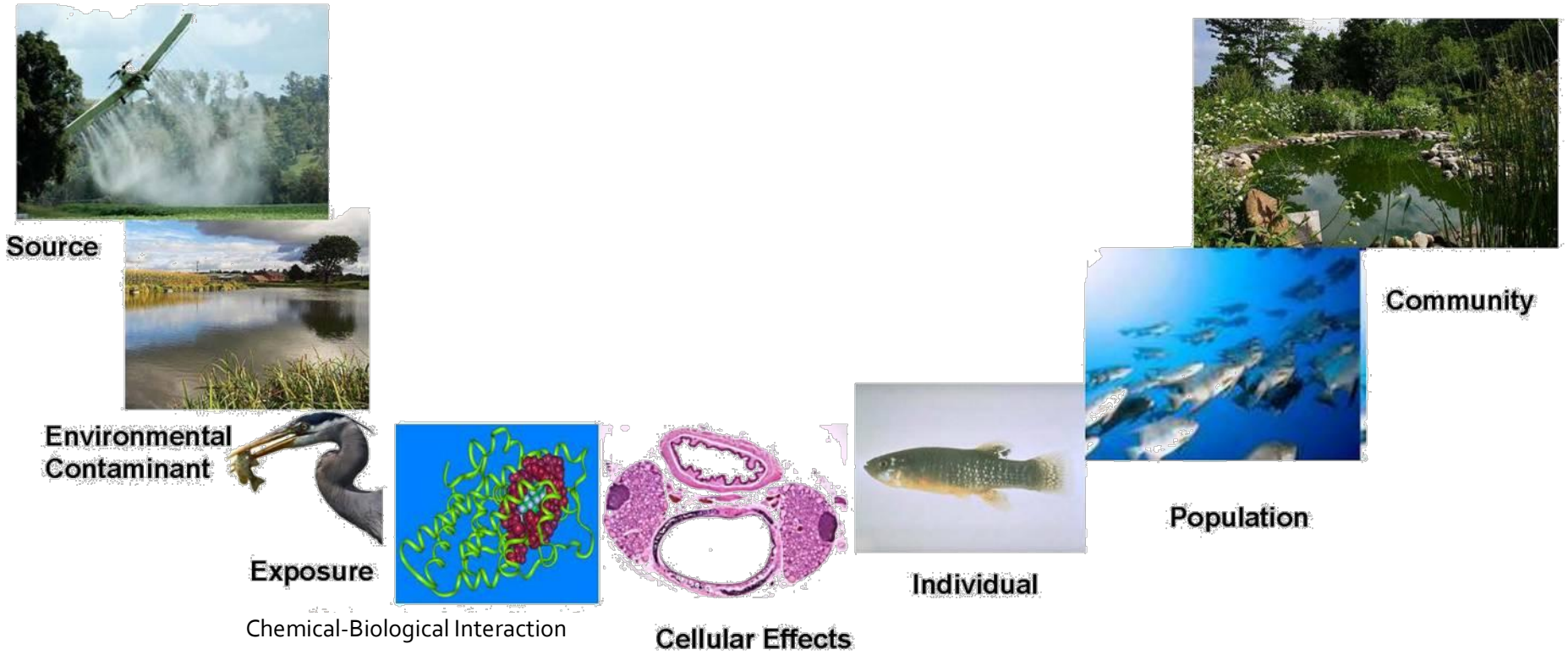
Tier 2

- **Mammalian two-generation rat**
  - May be replaced by Extended F1-Generation
- **Amphibian growth/reproduction**
  - (Xenopus) [US lead, OECD validation program]
- **Fish multi-generation**
  - (Medaka) [US lead, OECD validation program]

EDSP Prioritization, Screening & Testing

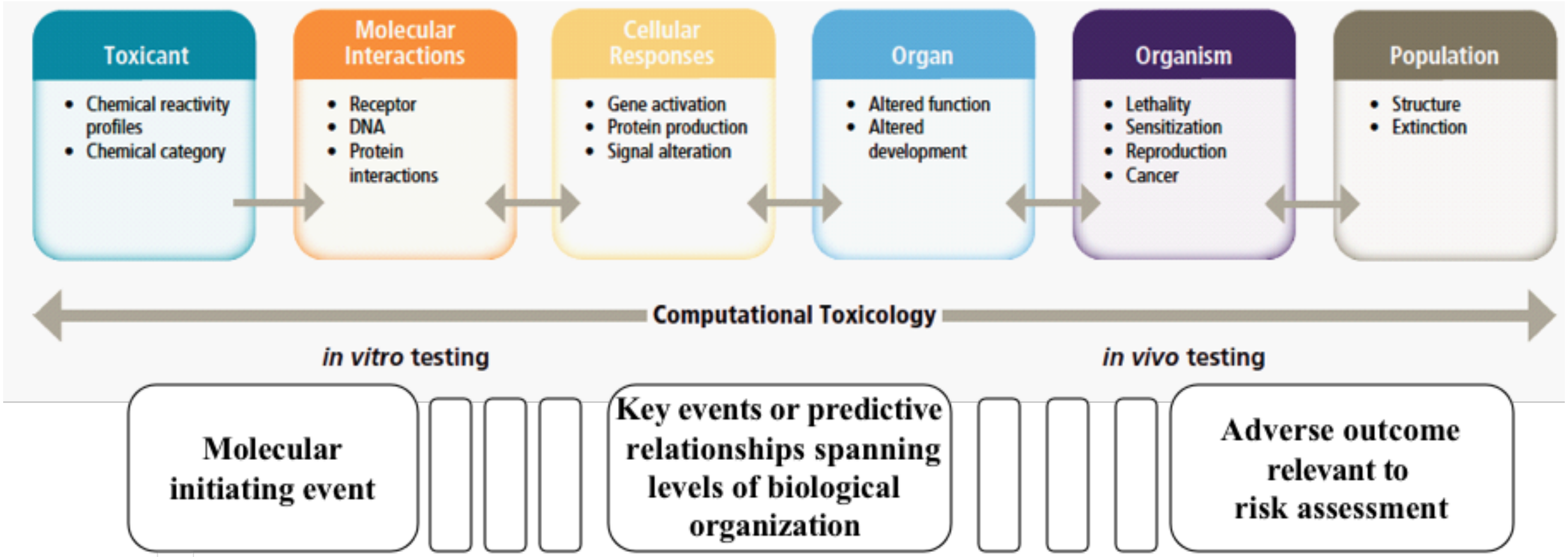


Adverse Outcome Pathways (AOP)



- Integrates chemical, biological and toxicological data relevant to exposure and effects
- Captures information across source-to-outcome continuum informs various steps of risk assessment process
- Part of EPA's strategy for Integrated Approaches to Testing and Assessment (IATA) and Endocrine Disruption Screening Program (EDSP)

Adverse Outcome Pathways



Endocrine AOP Concepts

- **Chemicals can activate multiple MIEs with varied outcomes across different life stages and taxa**

- **Pathway concept helps to show:**
  - Association of endpoints and tools with outcomes of regulatory interest
  - Provide case studies to guide weight of evidence assessments

- **Provides practical utility and decision criteria using high throughput screening (HTS) assays and computational tools, combined with conventional tools to characterize endocrine disruption potential**

- **AOP for E, A, and T and relevant case studies could be used to develop an overall guidance document for the application for AOPs**

EDSP Relevant AOP

- **Estrogen, Androgen and Thyroid (EAT)**

- **EDSP prioritization and assessment requires**
  - Measurement or prediction of in life dose-response
  - Monitoring or prediction of real-world exposures

- **Consistent with peer-review recommendations on use of Physical Chemical Properties, QSAR/HTS, and Exposure Predictions**

- **EDSP is focused on developing high throughput, AOP methods to prioritize targeted testing**

Application of AOPs for Endocrine Disruption Screening

- **Utilize AOPs for prioritizing and screening chemicals for their potential to interact with endocrine pathways leading to adverse effects**
  - Estrogen (E), androgen (A), and thyroid (T) pathways
- **Integrative approach**
  - Key measurement endpoints are considered in relation to the assessment tools (i.e., assays, computer models and test guidelines) relevant to the US EDSP
  - Measurement endpoints include molecular initiating event (MIEs) as well as intermediate and terminal events along the recognized pathways
  - AOP linkage of relevant measures and tools assists in the interpretation and assessment of chemicals for potential interaction and adverse consequence

Estrogen AOP

Study Type: HTS in vitro & in silico | Tier 1[1] | Tier 2[2]

Endpoint	Chemical category	QSAR	ToxCast	ER Binding	ERTA	Aromatase	Steroidogenesis	Uterotrophic	F Pubertal	FSTRA	Rat 2-gen/EOGRT	MEOGRT	LAGDA	Quail	
Physicochemical	x	x													Toxicant
ER binding		x	x	x											Molecular Interactions
ER activation		x	x		x										
DNA Binding		x	x												
Cell proliferation			x												
Protein production					x					x		x		x	Cellular Responses
Biochemistry									x	x	x	x		x	
Steroidogenesis			x			x	x								
Organ weight								x	x	x	x			x	Organ
Histopathology									x	x	x	x	x	x	
Onset of Puberty									x		x	x		x	Organisms
Estrous Cyclicity									x		x				
Fertility/Fecundity									x	x	x	x		x	Population
Development									x	x	x	x	x	x	

[1]Tier 1 screening test guidelines (series 890)
[2]Proposed Tier 2 test guidelines (series ?)

Androgen AOP

Study Type:	HTS in vitro & in silico						Tier 1[1]			Tier 2[2]				
Endpoint	Chemical category	QSAR	ToxCast	AR Binding	Aromatase	Steroidogenesis	Hershberger	M Pubertal	FSTRA	Rat 2-gen/EOGRT	MEOGRT	LAGDA	Quail	
Physicochemical	x	x												Toxicant
AR binding		x	x	x										Molecular Interactions
AR activation		x	x											
DNA Binding		x	x											
Biochemistry							x	x	x	x	x		x	Cellular Responses
Steroidogenesis			x		x	x								
Organ weight							x	x	x	x			x	Organ
Histopathology								x	x	x	x	x	x	
Onset of Puberty								x		x	x		x	Organism
2° Sex characteristics									x		x		x	
Fertility/Fecundity								x	x	x	x		x	Population
Development								x	x	x	x	x	x	

[1]Tier 1 screening test guidelines (series 890)
[2]Proposed Tier 2 test guidelines (series ?)

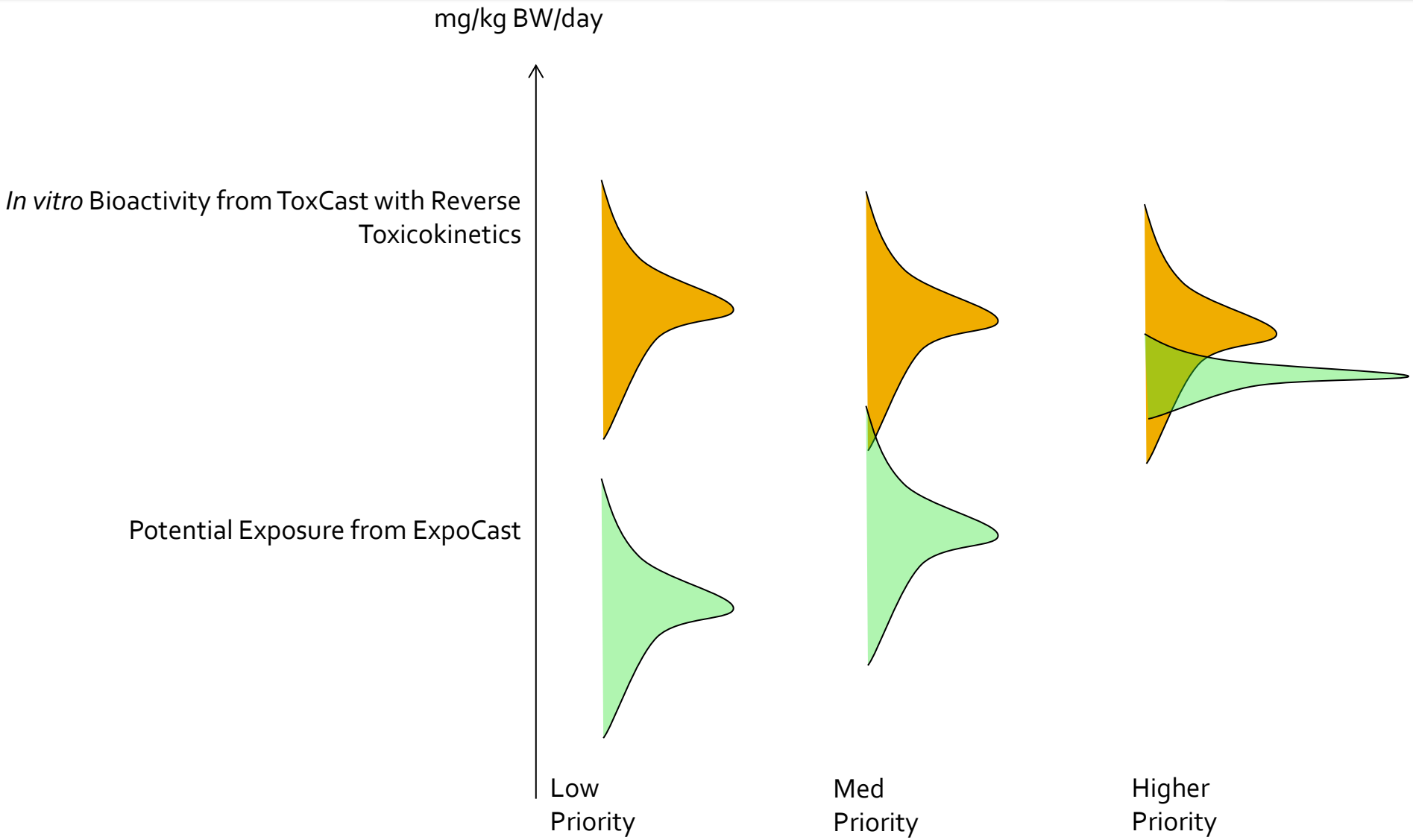
Thyroid AOP

Study Type: HTS in vitro & in silico | Tier 1[1] | Tier 2[2]

Endpoint	Chemical Category	QSAR	ToxCast	M Pubertal	F Pubertal	AMA	Rat 2-gen/EOGRT	LAGDA	Quail	
Physicochemical	X									Toxicant
TR activation			X							Molecular Interactions
Biochemistry				x	x		x		x	Cellular Responses
Organ weight				x	x		x		x	Organ
Histopathology				x	x	x	x	x	x	
Metamorphosis						x		x		Organism
Development						x	x	x	x	Population

[1]Tier 1 screening test guidelines (series 890)
[2]Proposed Tier 2 test guidelines (series ?)

Integrated Bioactivity-Exposure Relationships



Computational Toxicology and AOPs

- **HTS assays are being evaluated**
  - Build confidence they capture biological activities of interest for endocrine screening
  - Develop understanding of comparison to current, validated screening assays, proposed in vivo testing and other scientifically relevant information
- **Reference chemicals are being identified to validate individual HTS assays**
  - Results from HTS assays are being compared for concordance and redundancy to ensure adequate AOP coverage
- **HTS and other in vitro assays are being compared to in vivo assays to determine replacement value for screening the universe of chemicals:**
  - Reduce animal use
  - Provide efficient and robust approach protecting human and ecological health
- **Draft AOPs are being generated for E, A and T**