The Future of Endocrine Screening: An Animal Welfare Perspective

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Outline

- Animal use in the current EDSP design
- Need for a better approach
- Begin with modified tiered system + weight-of-evidence
- Move toward pathway-based approach

Tier 1 Animal Use

Assay	# animals/test
In vitro	
Estrogen Receptor Binding – rat cytosol	13[a]
Androgen Receptor Binding - rat cytosol	10[a]
Estrogen Receptor Transcriptional Activation	—
Aromatase Recombinant	—
Steroidogenesis H295R	—
In vivo	
Uterotrophic	18
Hershberger	48[b]
Male Pubertal	45
Female Pubertal	45
Fish Short-term Reproduction (♂&♀)	72
Amphibian Metamorphosis	320
Total	**571**

[a] for receptor extract

[b] the TG assumes both agonist and antagonist methods are performed

Putative Tier 2 tests

Assay	# animals/test
Mammalian 2-Generation	1600 - 2400
Amphibian 2-Generation	400 P, plus progeny
Avian 2-Generation	1024
Fish Lifecycle	~1000*
Total	**> 4,000**

*number varies by species

Phase I

Data submitted and generated for 54 chemicals in Phase I: OSRI was submitted for all or some assays for 47 chemicals*

Assay	Assays in Initial Test Plans	Assays in Final Test Plans	# Animals Initial Test Plans	# Animals Final Test Plans
In vitro				
ER Binding	2	37	26	468
AR Binding	2	37	20	360
ERTA	3	35	-	-
Aromatase	10	40	-	-
Steroidogenesis	17	40	-	-
Subtotal	34	187	46	828
In vivo				
Uterotrophic	12	35	216	612
Hershberger	16	31	768	1,632
Male Pubertal	3	44	135	1,440
Female Pubertal	4	43	180	1,710
Fish Short-term Reproduction	21	26	1,512	3,168
Amphibian Metamorphosis	15	32	4,800	13,120
Subtotal	**71**	**211**	**7,611**	**21,682**
Other 7 chems	**77**	**77**	**3,997**	**3.997**
Total	**182**	**501**	**11,508**	**26,507**
Total if all testing done for 54 chemicals				**30,834**

* Bishop, Sullivan and Willett, submitted

Acceptance of existing information:

Generally in cases of identical or nearly identical information to that provided by a Tier 1 test

Or indicated positive result

- Same study showing negative result was rejected

Weight-of-evidence inconsistently applied

* Bishop, Sullivan and Willett, submitted.

Need for a better approach

- Current program design is resource, labor and animal-intensive
- Provides much data, only some of which may have have regulatory use
- Provides insufficient opportunity for chemically-relevant tailoring
- Does not link mechanism to adverse outcome
- Is not predictive
- Does not fully cover “endocrine” effects

First step: a more refined tiered system

Initial tier takes into account all existing information, including physicochemcial

Second tier assesses potential mechanisms of action

Following tiers address potential effects in more complex systems, on multiple modes of action

Highest tiers address adverse outcome and dose-response

WoE with clearly articulated criteria applied at each tier

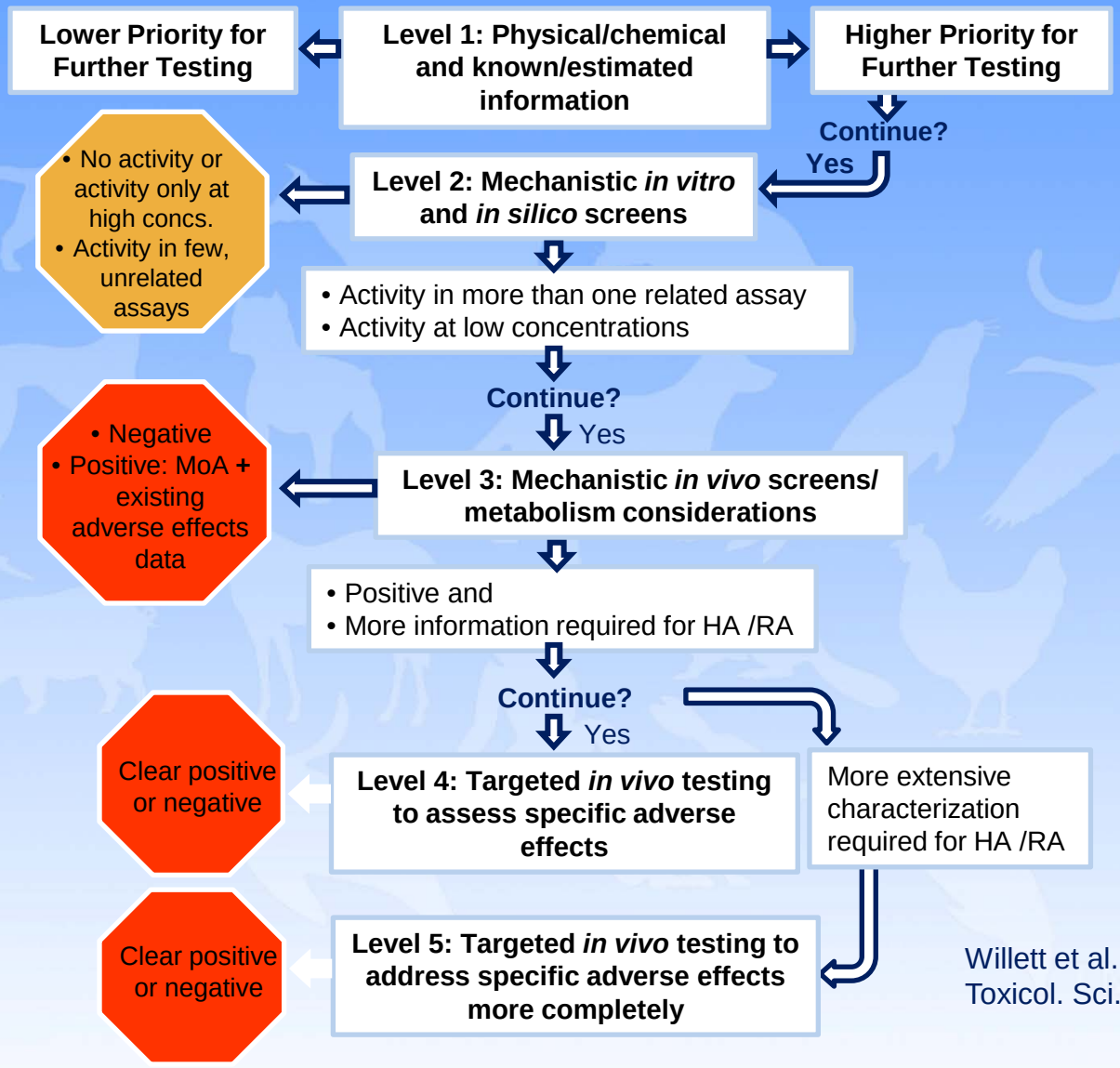
- Defines positive and negative cut-offs at the outset for each test and each Tier or Level
- Results are used to design the strategy for further testing

Strategy is designed to address information needs

OECD Conceptual Framework

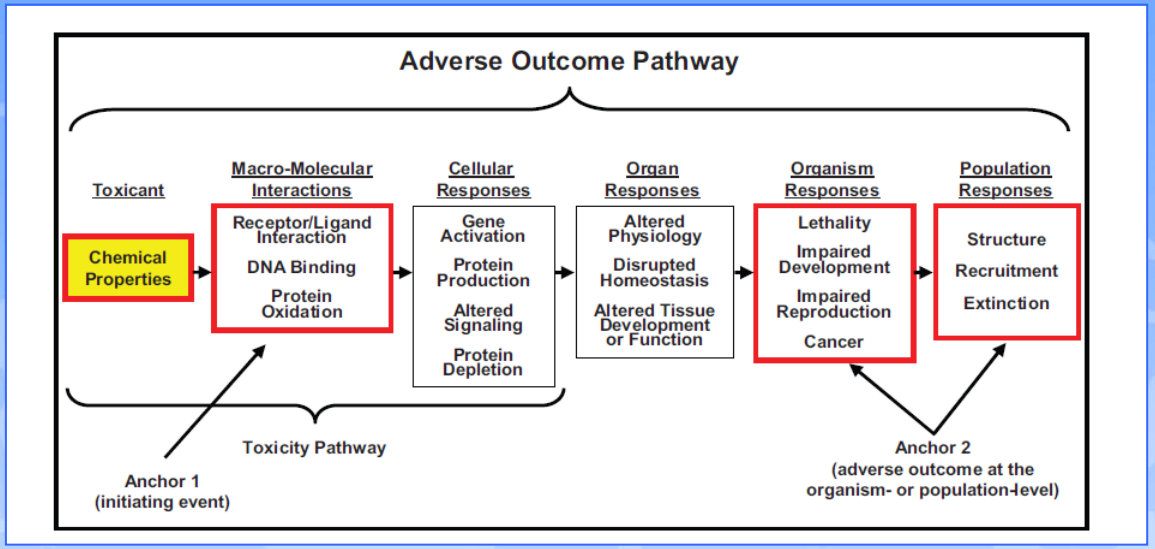
Level 1	**Sorting and prioritization based on existing information** •physical and chemical properties •exposure •available toxicological data
Level 2	***In vitro* assays providing mechanistic data** •QSARs •binding and transcription assays •aromatase, steroidogenesis, thyroid function •high-throughput screens
Level 3	***In vivo* assays addressing single mechanisms and effects** •uterotrophic •Hershberger •amphibian metamorphosis •fish VTG (e.g. TG 230)
Level 4	***In vivo* assays addressing multiple mechanisms and effects** •enhanced 407 •male and female pubertal assays •adult intact male •fish short-term reproductive assay (e.g. TG 299)
Level 5	***In vivo* assays providing data on effects from endocrine + other mechanisms** •1 and 2 generation reproduction in rodents, birds, fish

Refined tiered system with integrated assessment



Willett et al. 2011. Toxicol. Sci.123(1):15–25.

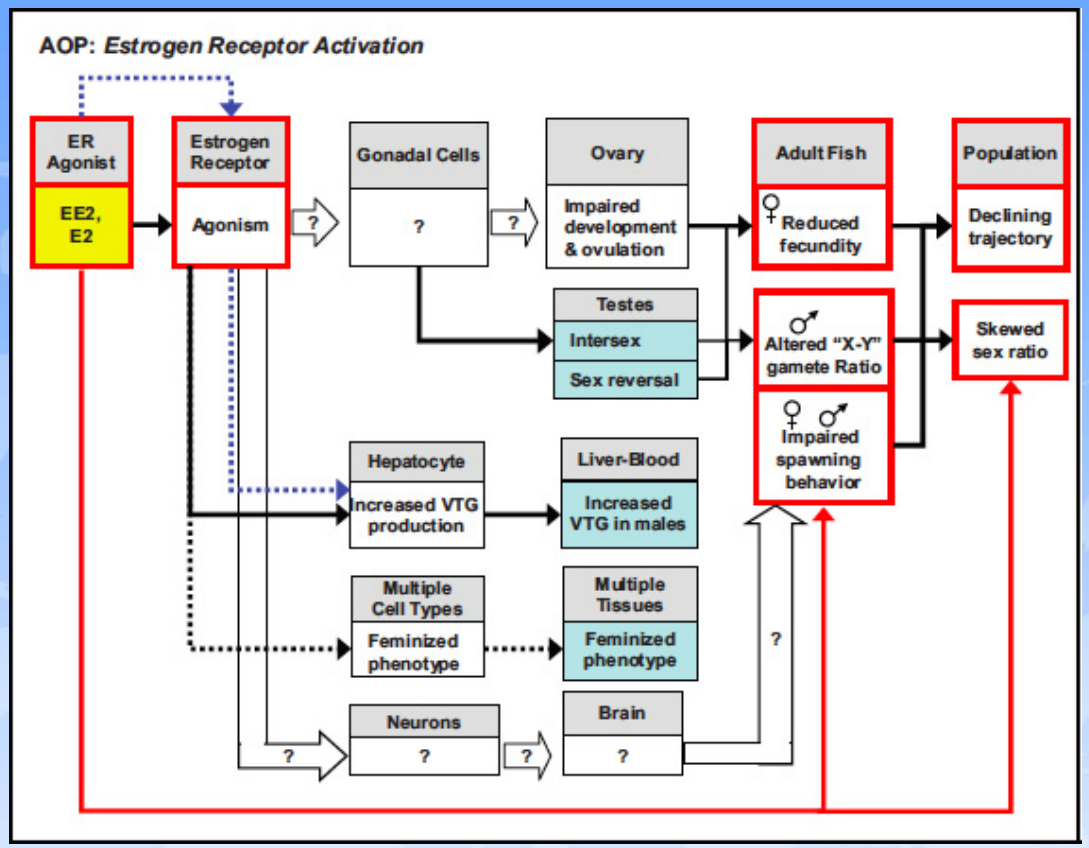
The future of EDSP: pathway-based approach



From: Ankley et al. Environ.Toxicol.Chem. 2010. 29 (3): 730–741.

A **conceptual construct** that portrays existing knowledge of linkages between a **direct molecular initiating event** and an **adverse outcome** at a level of biological organization relevant to risk assessment

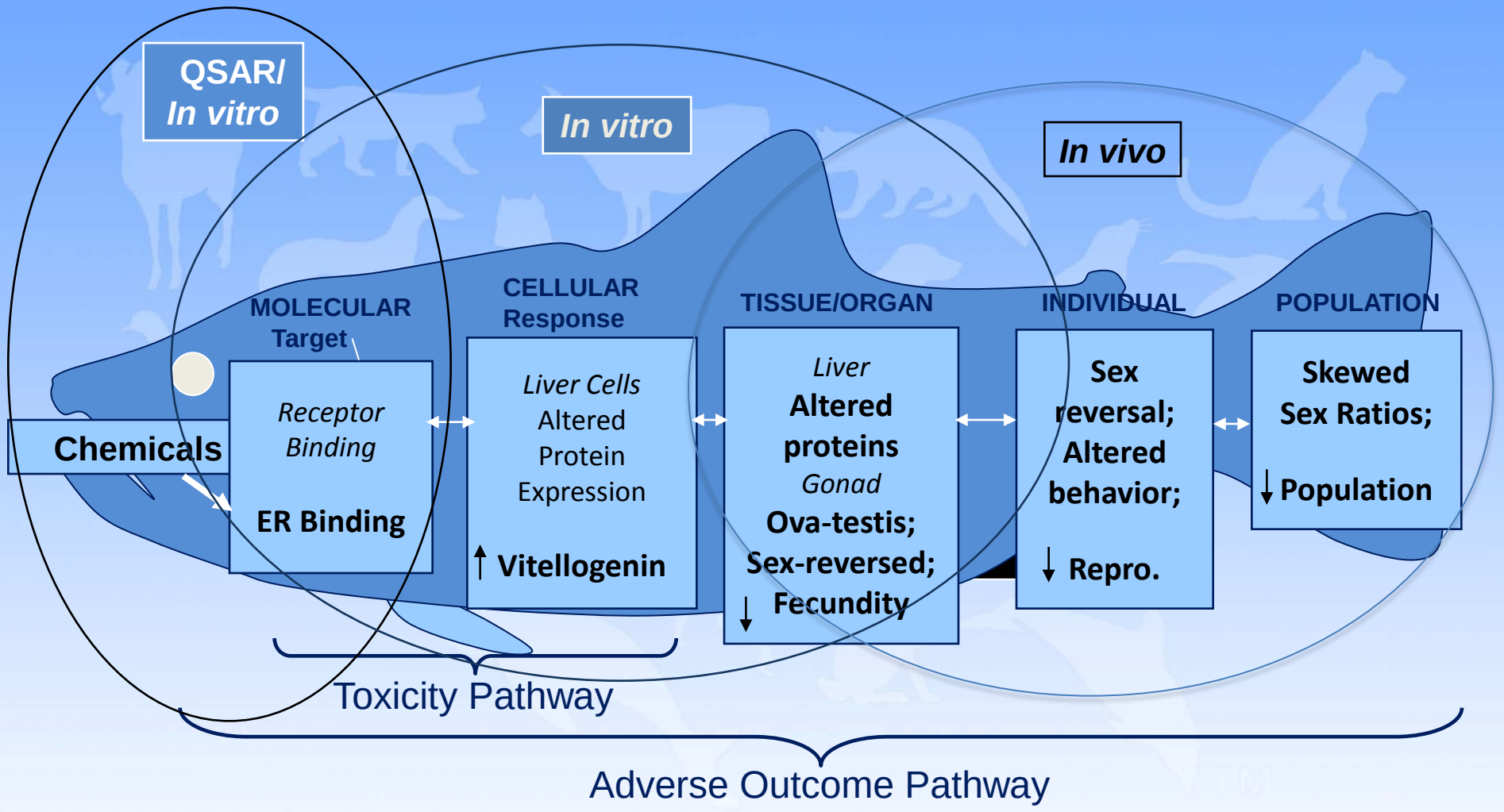
ER-mediated reproductive impairment



Developed from:

- an understanding of the MIE plus
- a need for a strategy for measuring endocrine-mediated effects.
- Includes use of biomarker (vitellogenin) that is (in the case of females) or is not (in the case of males) directly linked to the adverse outcome

ER-mediated reproductive impairment



P. Schmieder, McKim conference 2008.

ER and AR pathway efforts

US EPA:

- AOP development
- HTS and other in vitro assays

The Hamner Institutes: “Tier 1 and Done”

CAAT: Pathways of Toxicity

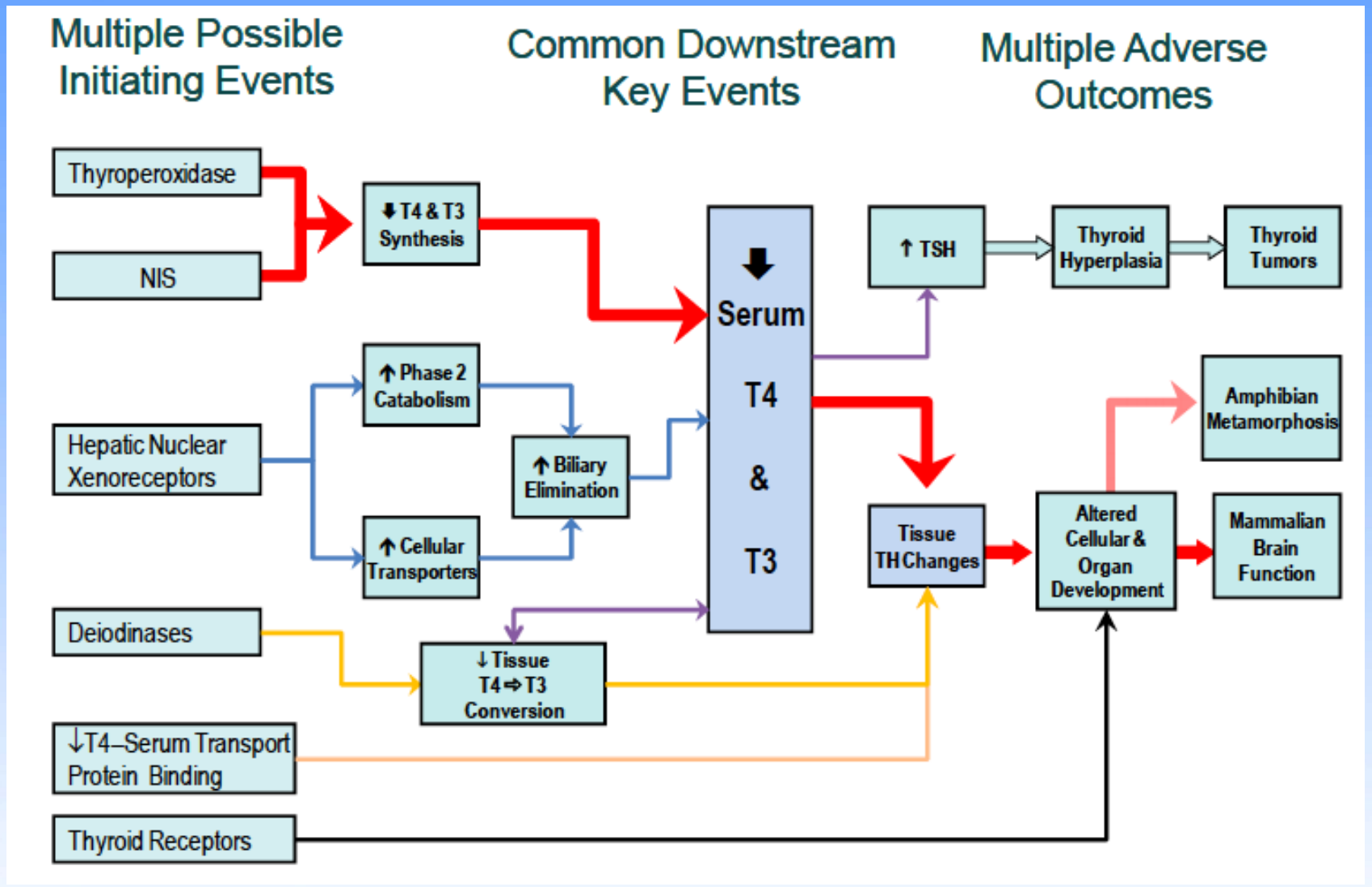
OECD Validation Management Group – Non-animal

- Binding and TA assays

OECD Advisory Group on Molecular Screening and Toxicogenomics

- AOP development

Thyroid hormone pathway



Crofton, K. US EPA. Presented at DC area SOT, May 2012.

Thyroid pathway efforts

Development of AOPs and relevant assays

- OECD Thyroid Scoping Effort Expert Group
- OECD Validation Management Group – Non-animal
- OECD Advisory Group on Molecular Screening and Toxicogenomics
- US EPA

Other necessary elements

- Build Biological and adverse-outcome "pathways"
  - OECD integration of AOPs into the Test Guidelines program
  - Guidance
- Improve predictive tools
  - NIH National Center for Advancing Translational Sciences
  - EPA's Computational Toxicology Research
  - OECD QSAR tool box
  - Hamner Institute
- Develop assessment systems for complex endpoints
  - Reconstructed tissues and organ systems
    - Human skin, eye, lung
    - Liver-on-a-chip
    - Stem-cell derived
- Integrate absorption, metabolism and distribution information
  - QSAR QIVIVE -
  - Liver cells, tissues, extracts, reconstructed tissues
- Integrated databases and "knowledge bases"
  - **ACToR and MetaPath:** EPA – all available chemical toxicity data on over 500,000 environmental chemicals searchable by chemical name and structure
  - **Kegg pathway database**: collection of manually drawn pathway maps representing current knowledge on the molecular interaction and reaction networks
  - **AOP Wiki:** EPA-European Commission joint project to house developing AOPs

Conclusions

The current EDSP design could be improved in terms of efficiency and utility of data generated

Could begin with:

- a more refined organization of tiered tests
- combined with an iterative weight-of-evidence analysis

As AOPs and tests are developed, move toward pathway-based approach

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H E S I

Thank You

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