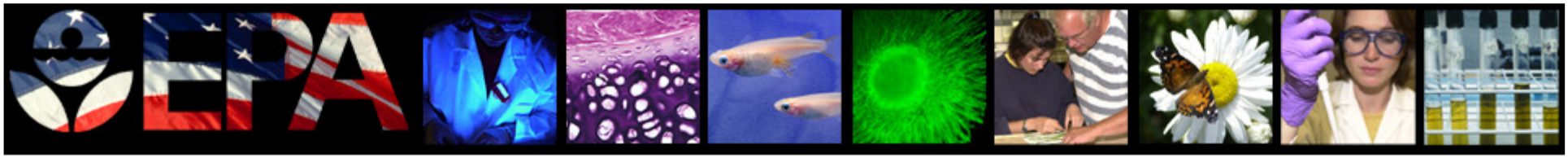

Consideration of Reproductive/Developmental Mode of Action Data from Laboratory Animals in the Risk Assessment of Environmental Chemicals

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The views expressed are those of the speaker and do not necessarily reflect the views or policies of the U.S. Environmental Protection Agency.

United States Environmental Protection Agency

Overview

- MOA definitions
- MOA framework
- Availability of MOA data
- Potential uses of MOA information
- Areas for further consideration

Mode-of-Action (MOA) Data

Mode-of-action is defined as the sequence of key cellular and biochemical events (measurable parameters) that result in a toxic effect, while *mechanism of action* implies a more detailed understanding of the molecular basis of the toxic effect. Complete mechanism of action information is rarely available and is not required for human health risk assessment. (*Seed et al. 2005)*

Mode-of-action key events

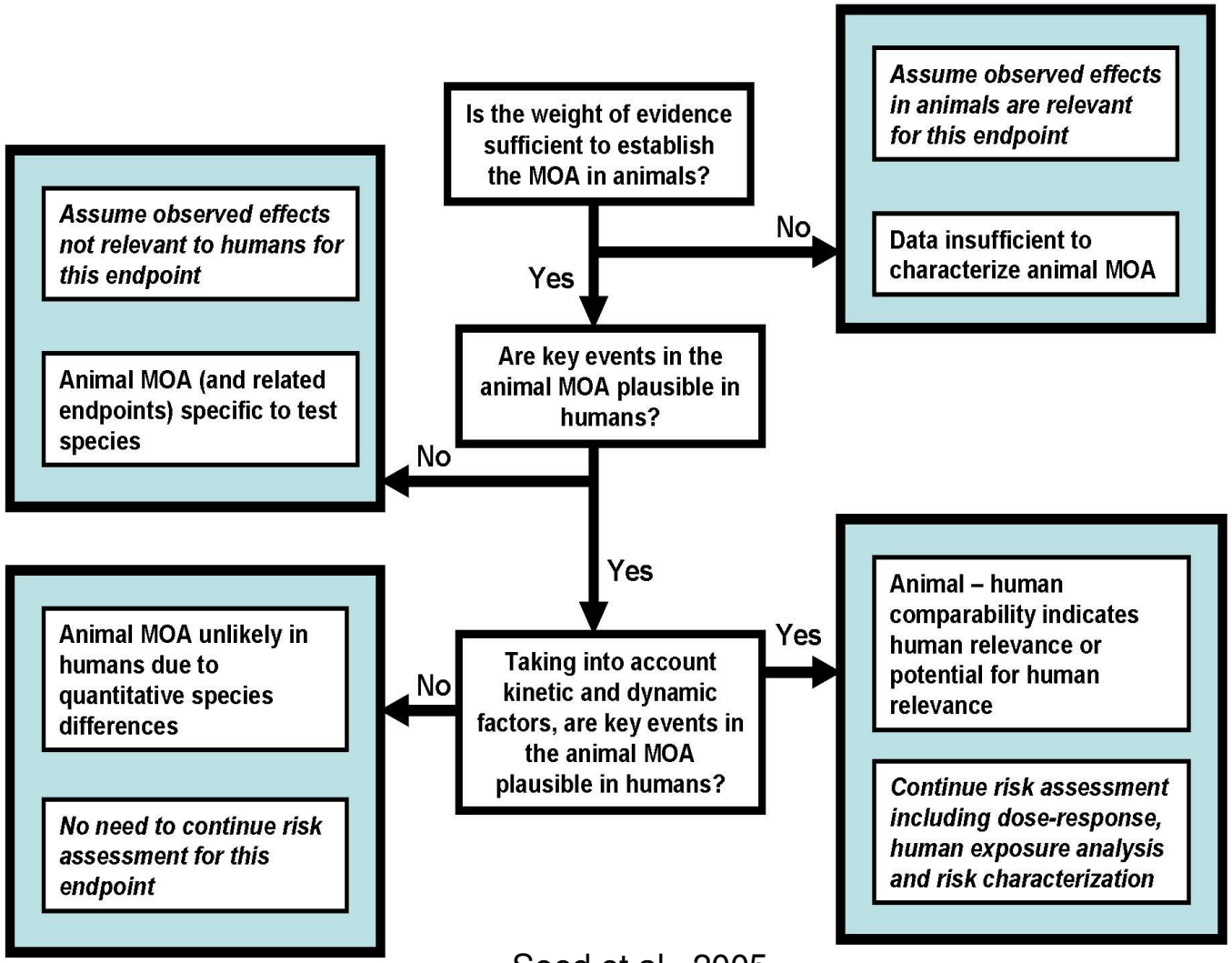
- Must be linked (correlative or causative) to adverse outcome
- Lack of key event = no toxicity

Framework for Evaluating Human Relevance for Carcinogenic MOAs

- Weight of evidence for a toxicological response in experimental animals
- Postulated mode of action (theory of the case)
- Experimental Support for Key events
  - Concordance of dose-response relationships
  - Temporal association
  - Strength, consistency and specificity of association of toxicological effect with key events
  - Biological plausibility and coherence
- Other possible modes of action
- Uncertainties, inconsistencies, and data gaps

EPA 2005; IPCS

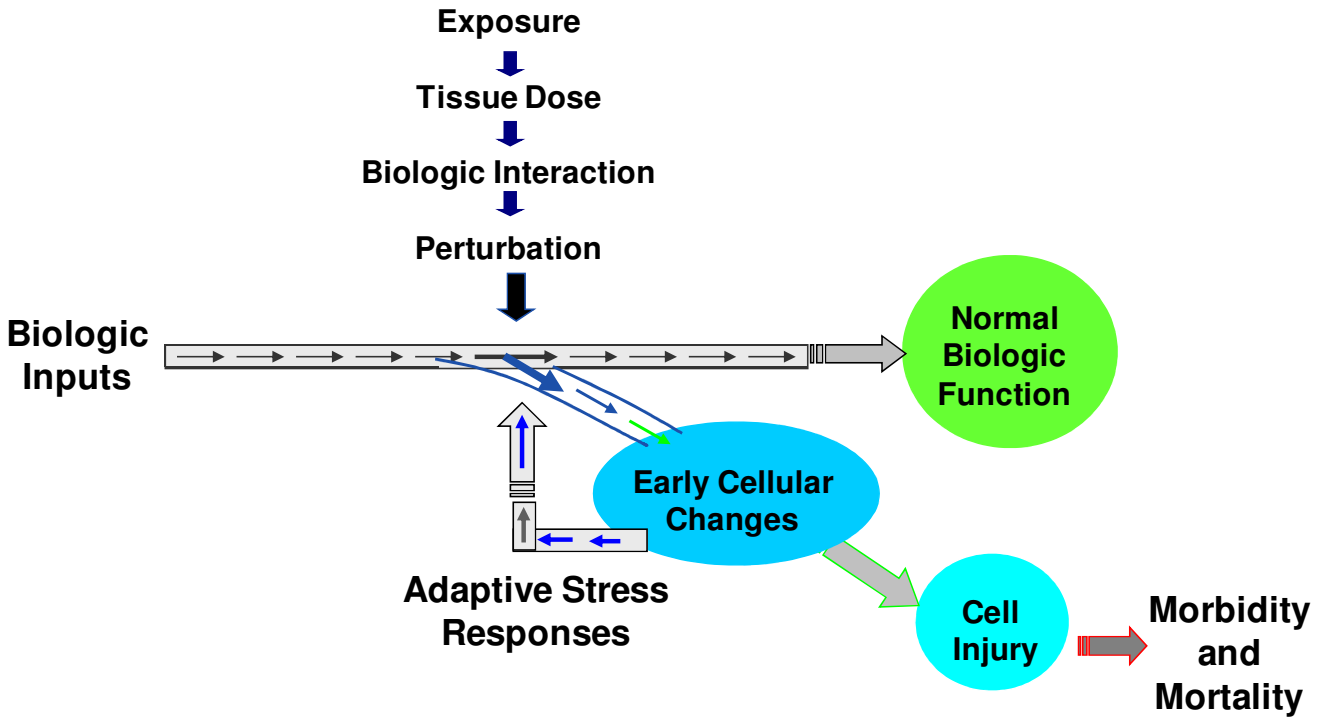
MOA Framework for Non-Cancer Endpoints



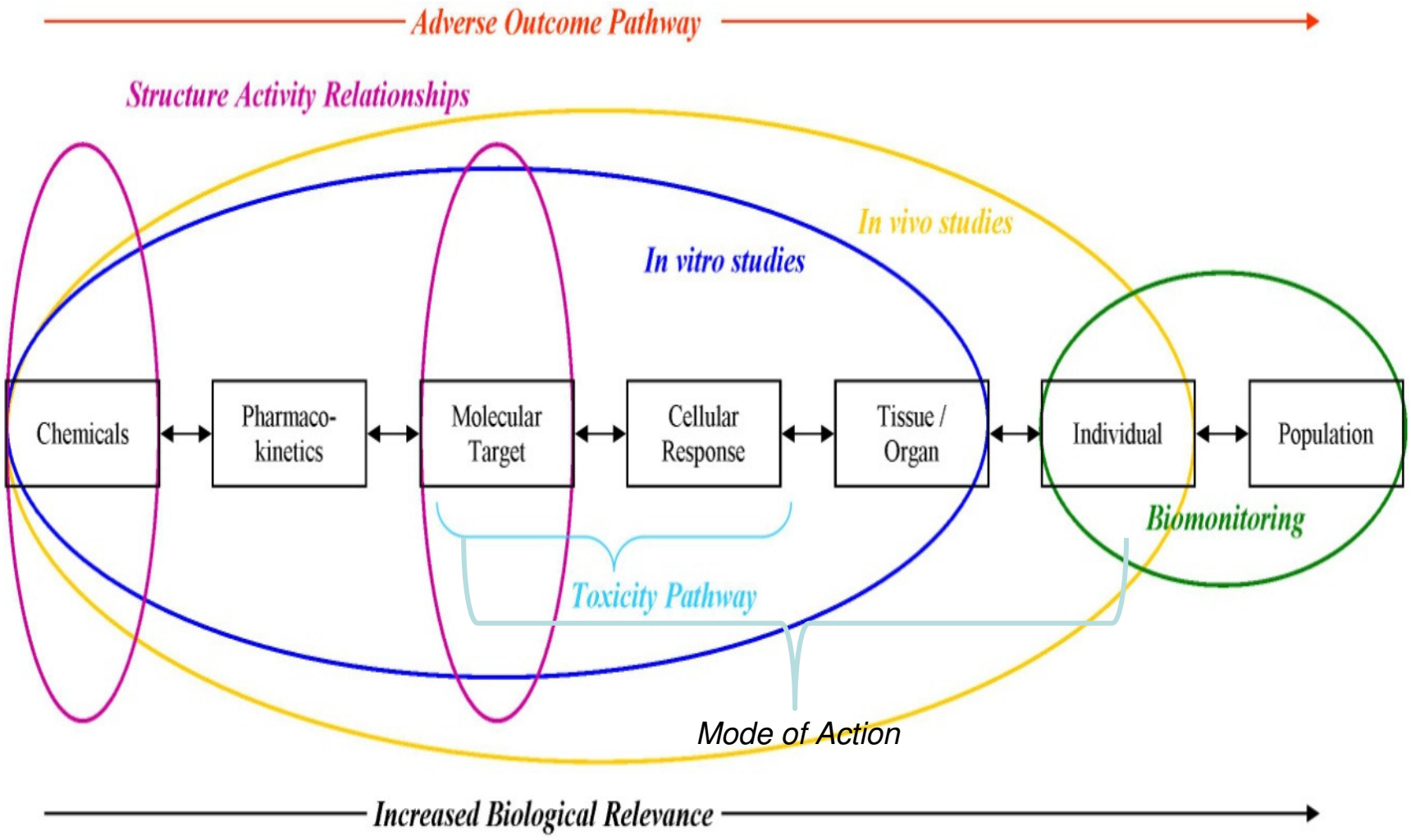
Seed et al., 2005

Toxicity Pathway

- **Definition:** Cellular response pathways that, when sufficiently perturbed, are expected to result in adverse health effects are termed *toxicity pathways* (NRC 2007).



NRC, 2007


Adverse Outcome Pathway
Structure Activity Relationships
In vivo studies
In vitro studies
Chemicals
Pharmaco-kinetics
Molecular Target
Cellular Response
Tissue / Organ
Individual
Population
Biomonitoring
Toxicity Pathway
Mode of Action
Increased Biological Relevance

Availability of MOA Data for Environmental Chemicals

- Evaluation/testing for MOA is not required
  - Standard guideline studies seldom identify MOA
  - MOA data (if they exist) are often found in published literature
- Risk assessment can be conducted without this information
- It is more likely to have informative MOA data for pharmaceuticals
  - Stakes are higher due to direct administration of a pharmacological dose to humans; avoid adverse outcome and potential litigation

Potential Uses of MOA data in Risk Assessment for Environmental Chemicals

- Establish human relevance of a treatment-related response in animals
  - Cross-species extrapolation
  - Adjust level of concern for toxicological effects
- Provide information regarding uncertainties in the risk assessment (adjust UFs)
- Justify selection of endpoints for reference value determination (examples: DBP upstream effect, thyroid/DNT issues)
- Contribute to WOE for hazard characterization (example: TCE cardiac malformations)
- Provide information for some cumulative risk assessment decisions
- High throughput data (e.g., in vitro or –omic data)
  - Establish research priorities
  - Refine study designs
  - Contribute to characterization of mechanistic pathways of toxicity

Example: Use of MOA Data in RfV Determination

Proposed Use of Upstream Effects for Dibutyl Phthalate

Fetal Androgen Insufficiency

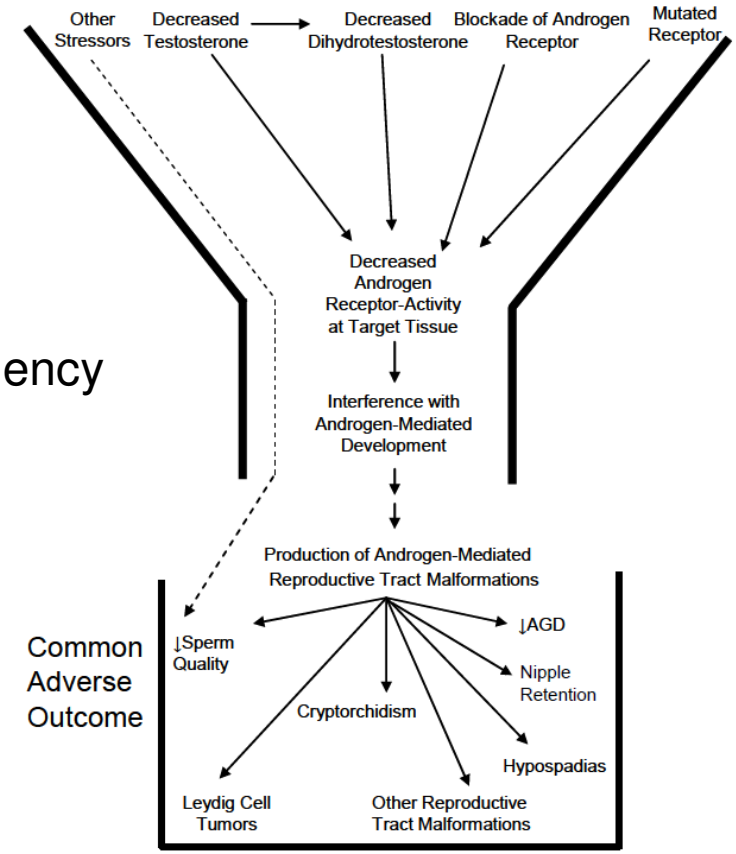


FIGURE 3-4 Fetal androgen insufficiency and common adverse outcomes.

NRC, 2008

Example: Use of MOA Data in RfV Determination

Proposed Use of Upstream Effects for Dibutyl Phthalate

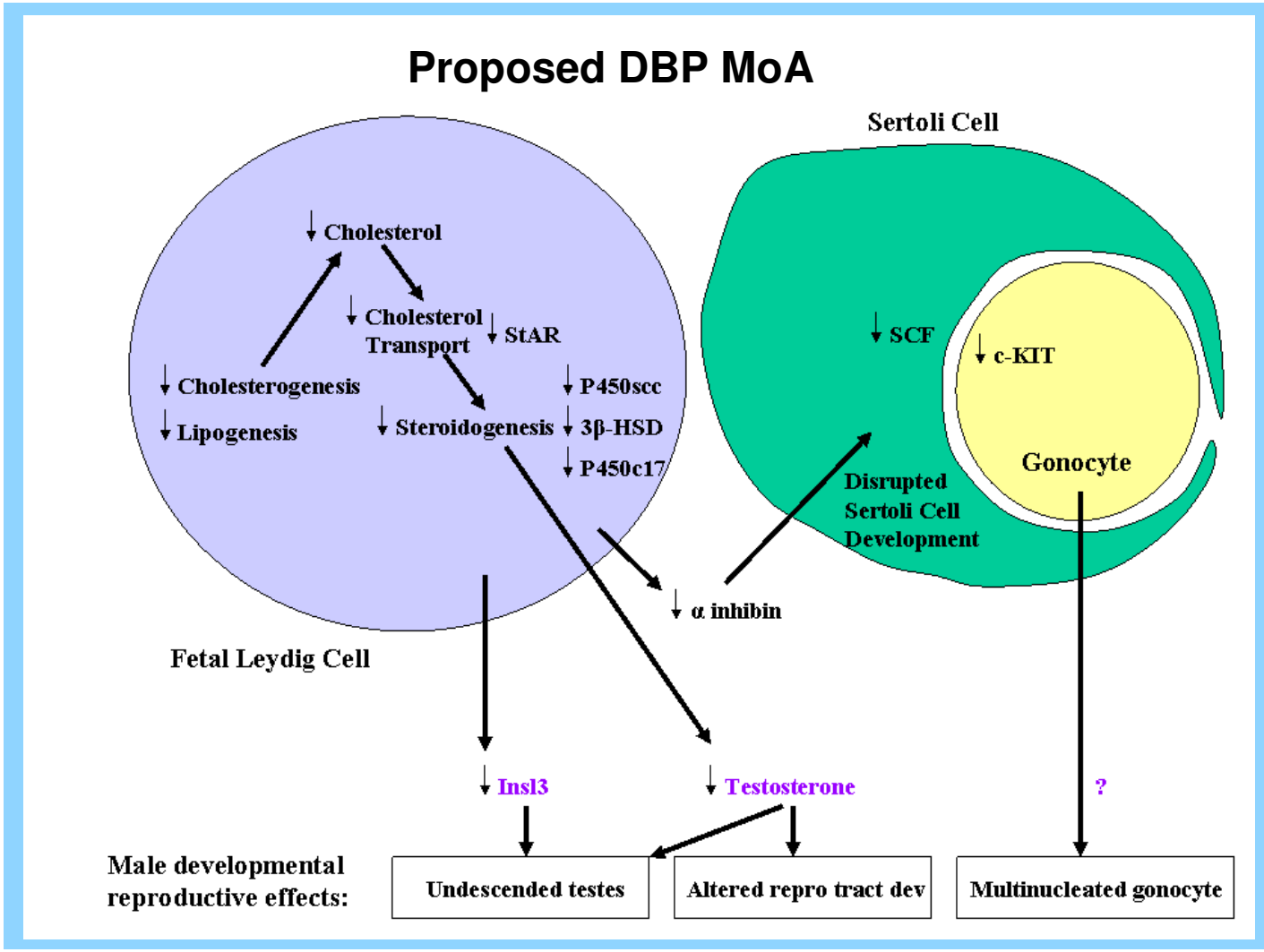
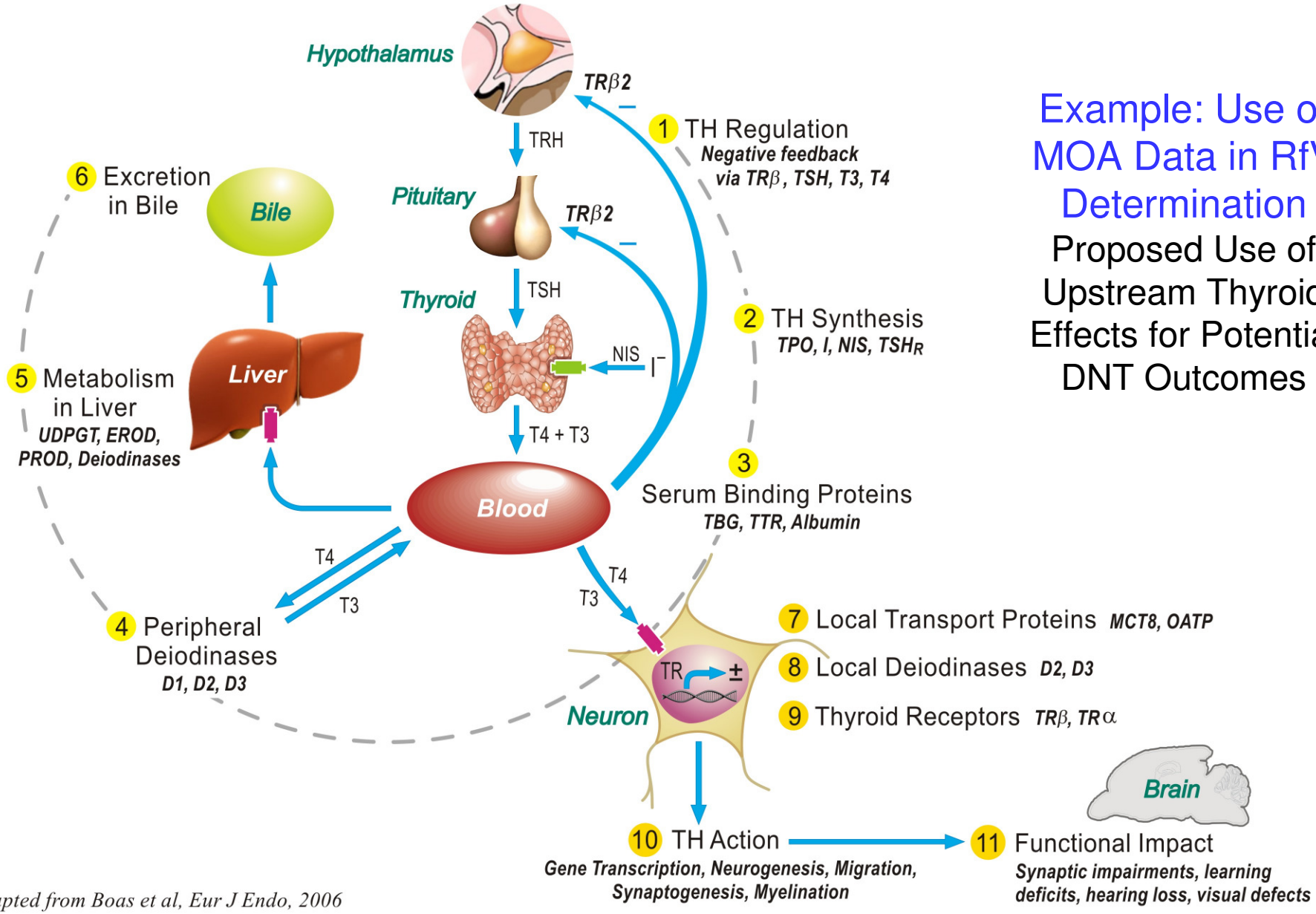


Figure adapted from Barlow et al. (2003), Liu et al. (2005), Shultz et al. (2001), Thompson et al. (2004), and Wilson et al. (2004). Based on male reproductive developmental toxicity and toxicogenomics studies. Some genes and pathways found to be altered are included. Purple lettering indicates proposed modes of action.

Possible Sites of Action of Environmental Contaminants on HPT Axis



Adapted from Boas et al, Eur J Endo, 2006

Example: Use of MOA Data in RfV Determination

Proposed Use of Upstream Thyroid Effects for Potential DNT Outcomes

Example: MOA Contribution to WOE for Hazard Characterization

Trichloroethylene Cardiac Defects

- Limited number of epidemiology studies showed increased risk of cardiac defects associated with TCE exposure
  - Confounders, especially environmental co-exposure
  - Some studies (with small number of cases) showed no association
- Animal developmental toxicology data
  - A number of studies identified cardiac malformations in rats
    - Studies were performed by the same research group over a number of years
    - Questions raised about methods used and statistical analysis
  - A number of studies did not identify cardiac malformations
    - Some of these studies used methods that may not have detected cardiac defects
    - One study was conducted using methods of the research lab and results were negative

Example: MOA Contribution to WOE for Hazard Characterization

Trichloroethylene Cardiac Defects – cont.

- MOA information supporting the potential association of TCE exposure with disruptions in cardiac valve formation
  - In vitro studies
    - Altered endothelial nitric oxide synthase (which generates nitric oxide that has an important role in normal endothelial cell proliferation and hence normal blood vessel growth and development)
    - Perturbation of proteins *(e.g., Serca2a* and *Ryr2*) involved in regulation of intracellular Ca^{2+} which could lead to morphogenic consequences in the developing heart
  - In ovo studies – Consistently showed treatment-related alterations in endothelial cushion development that could plausibly be associated with defects involving septal and valvular morphogenesis in rodents and chickens

Events in Cardiac Valve Formation in Mammals and Birds

Stage and event	Structural description
Early cardiac development	The heart is a hollow, linear, tube-like structure with two cell layers. The outer surface is a myocardial cell layer, and the inner luminal surface is an endothial layer. Extracellular matrix is between the two cell layers.
Epithelial-mesenchymal cell transformation	A subpopulation of endothelial sells lining the atrioventricular canal detaches from adjacent cells and invades the underlying extracellular matrix. Three events occur •Endothelial cell activation (avian stage 14) •Mesenchymal cell formation (avian stage 16) •Mesenchymal cell migration into the extracellular matrix (avian stages 17 and 18).
Mesenchymal cell migration and proliferation	Endothelial-derived mesenchymal cells migrate toward the surrounding myocardium and proliferate to populate the atrioventricular (A-V) canal extracellular matrix.
Development of septa and valvular structures	Cardiac mesenchyme provides cellular constituents for •Septum intermedium •Valvular leaflets of the mitral and tricuspid A-V valves. The septum intermedium subsequently contributes to •Lower portion of the interatrial septum •Membranous portion of the interventricular septum.

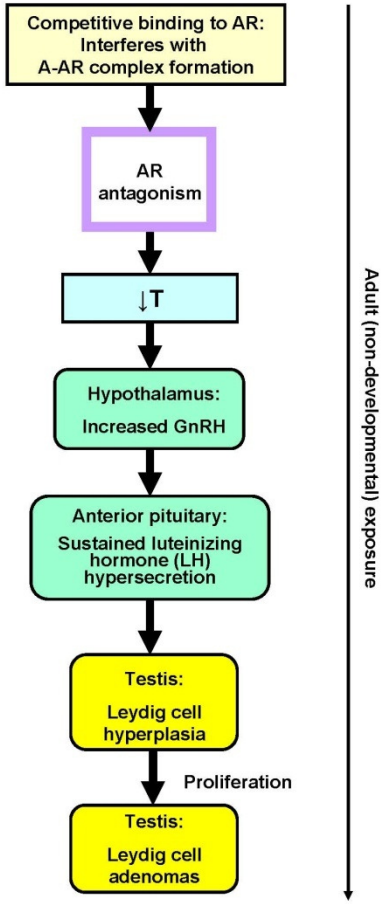
Review of MOA Frameworks
Key Areas for Further Consideration

- Determining sufficiency of evidence for defining a MOA or establishing human relevance
- Utilizing MOA data in characterizing additivity to background diseases, exposures and processes, including characterizing human variability in susceptibility
- Integrating information across multiple mechanistic pathways and incorporating systems level information into MOA analysis
- Harmonizing MOA information or mechanistic pathways across multiple outcomes
- Utilizing MOA information to inform quantitative considerations, e.g.
  - Extrapolation across species, dose, or exposure-windows
  - The use of precursor or biomarker data in dose-response assessment
  - The quantification of uncertainty estimates

Guyton et al., 2008

Example: Single vs. Multiple MOA

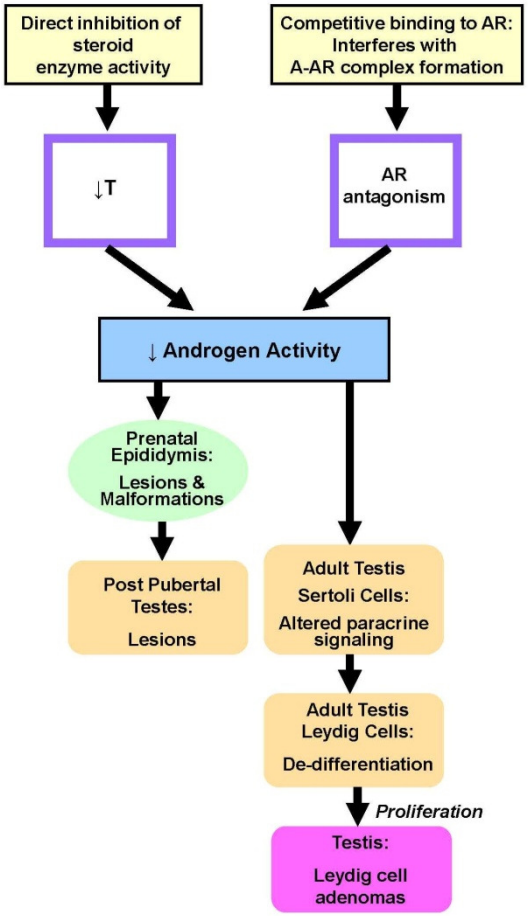
Proposed MOA for Linuron Leydig Cell Adenomas



Cook et al., 1993

- MOA proposed in 1993 for Leydig cell tumorigenesis that involves interruption of the HPT axis and sustained LH hypersecretion in adult (P) rats following long-term exposures to linuron
- Recent re-evaluation concluded that the data used to support the proposed MOA are limited, weak, and conflicting .

Proposed Dual MOA for Linuron Developmental Toxicity and Leydig Cell Adenomas



Gray et al., 1999
Wilson et al., 2009

- There is an extensive database of studies that support a hypothesized dual MOA (direct testosterone inhibition and competitive binding to the androgen receptor) for outcomes in the development of the male reproductive system in F1 rodents following prenatal exposures to linuron
- This proposed dual MOA is also relevant to the Leydig cell adenomas