

Evaluating AOP Evidence

Creating an Adverse Outcome Pathway in the AOP Wiki
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Outline/Objectives

Why/How we evaluate evidence for AOPs

- Background
- Components of Evaluation
 - – OECD Handbook/wiki
- Principles of Best Practice

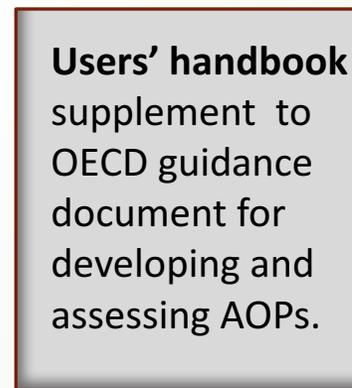
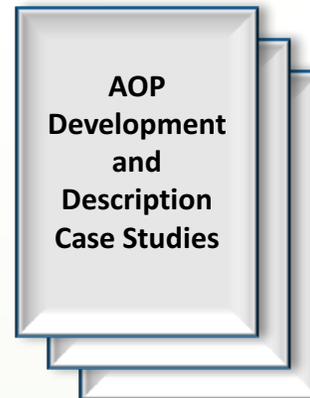
An introduction

Formalizing AOP Descriptions and Assessment to Support Regulatory Application

- OECD Guidance on Developing and Assessing AOPs (2013, 2014)
 - Conventions and terminology
 - Information content of an AOP description
 - **Weight of evidence (WOE)/confidence evaluation**



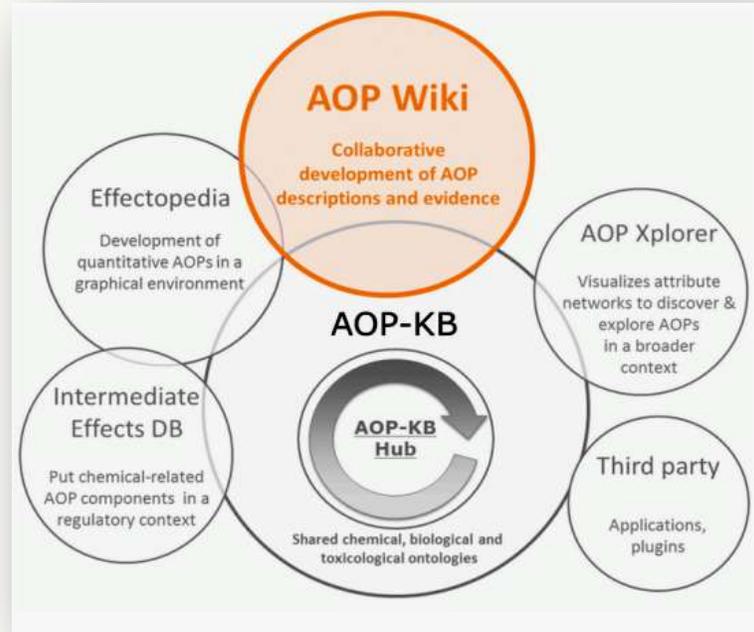
AOPWIKI.org



http://aopkb.org/common/AOP_Handbook.pdf

Addressing the Research-Regulatory Interface: The AOP Knowledge Base

OECD
AOP devt and
assessment (2012)
Test Guidelines
Hazard Evaluation



AOPKB.org
AOPWIKI.org

> 200 AOPs

Facilitating research collaboration:

- Avoiding duplicative effort
- Integration and analysis
- Building networks
- Accessible and searchable

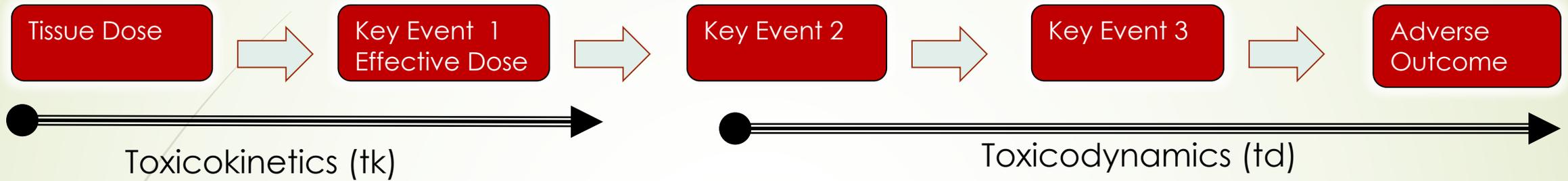
Addressing regulatory needs:

- Systematically organized
- Transparent, well documented
- Scientifically-defensible, credible



Identifying data gaps relevant to application

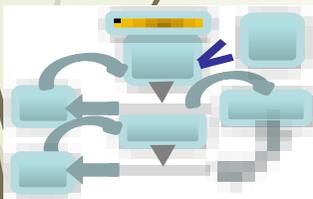
Mode of Action/Adverse Outcome Pathways



Chemical specific
absorption, distribution,
metabolism, excretion

Chemical agnostic biological
pathway

Adverse Outcome Pathway
(AOP)

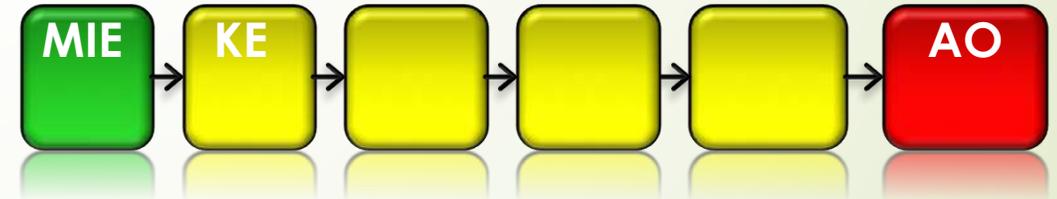


MOA
Analysis;
Biological
Plausibility in
Epi Studies

Integrated
Testing

Monitoring
of
Environment

AOPs



Background – WOE Analysis for AOPs

- ▶ Draws on experience in mode of action (MOA) analysis for regulatory application
 - ▶ Modified for AOPs (non chemical specific biological pathway)
- ▶ Based on modified Bradford Hill (B/H) considerations
 - ▶ Initially introduced to assess causality of associations observed in epidemiological studies in humans
 - ▶ later adapted to impacts on wildlife (“ecoepidemiology”)
- ▶ Guidance expected to evolve as additional AOPs are developed and documented

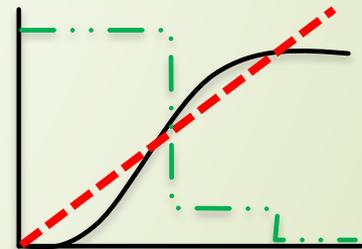
Weight of Evidence/Quantitation of KERs

Qualitative WOE

- To **simplify**, clarify and “codify” to the extent possible, qualitative WOE consideration addressing:
 - Focus (a limited no. of critical elements)
 - Including “patterns of empirical support”
 - Clarification of the nature of supporting data through:
 - defining questions
 - criteria & examples

Quantitation of KERs

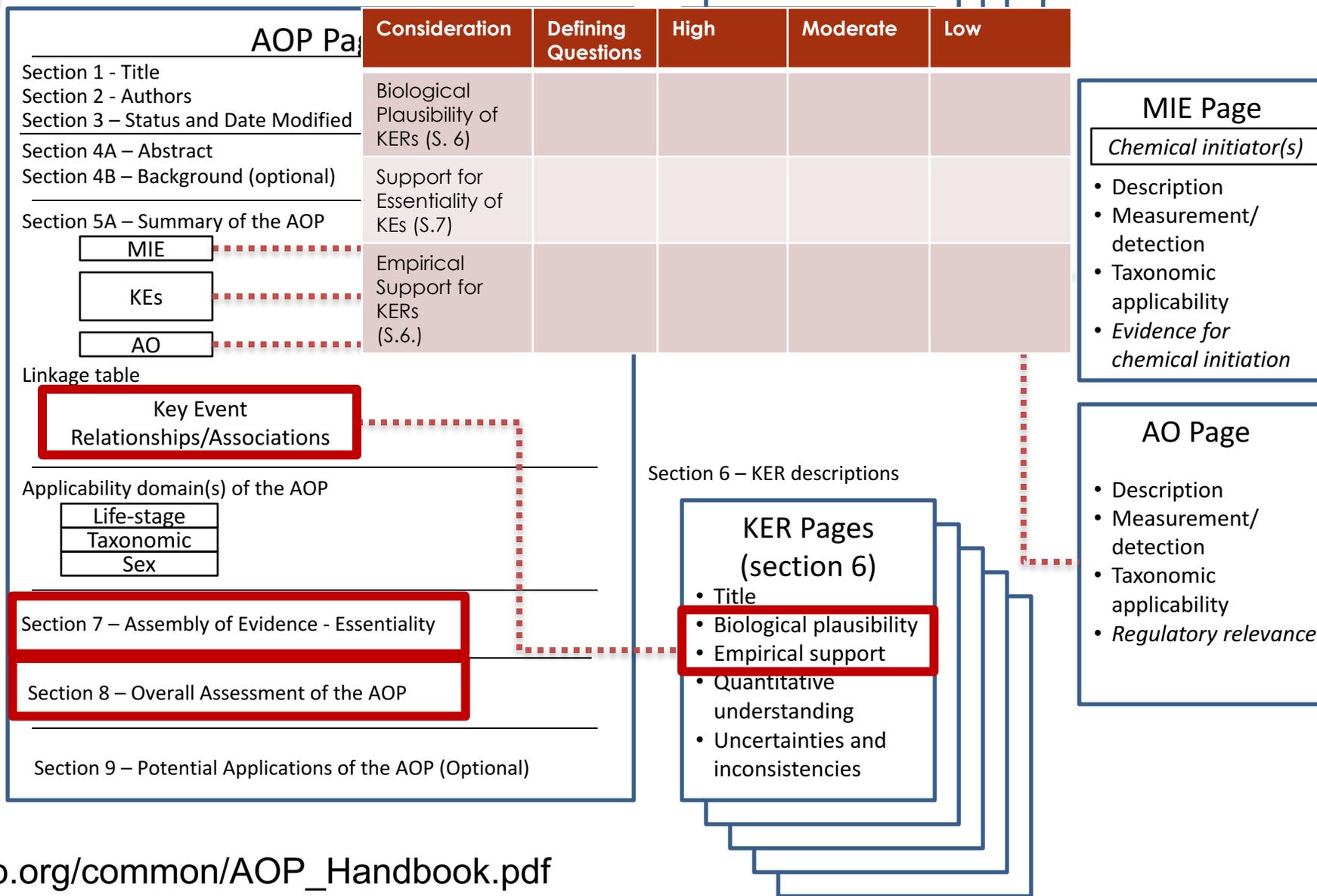
- quantitation of the KERs, as a basis for developing predictive response-response models



How much change in KE_{up} is needed to evoke some unit of change in KE_{down} ?

Annex 1

Section 5b – MIE, KE, and AO descriptions



Annex I

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3. Empirical Support _b for KERs	Defining Question	High	Moderate	Low
	<p>Does KE_{up} occur at lower doses and earlier time points than KE_{down} and at the same dose of stressor, is the incidence of KE_{up} > than that for KE_{down}?⁶⁷.</p> <p>Are there inconsistencies in empirical support across taxa, species and stressors that don't align with expected pattern for hypothesized AOP?</p>	<p>Multiple studies showing dependent change in both events following exposure to a wide range of specific stressors. (Extensive evidence for temporal, dose-response and incidence concordance) and no or few critical data gaps or conflicting data</p>	<p>Demonstrated dependent change in both events following exposure to a small number of specific stressors and some evidence inconsistent with expected pattern that can be explained by factors such as experimental design, technical considerations, differences among laboratories, etc..</p>	<p>Limited or no studies reporting dependent change in both events following exposure to a specific stressor (i.e., endpoints never measured in the same study or not at all); and/or significant inconsistencies in empirical support across taxa and species that don't align with expected pattern for hypothesized AOP</p>
MIE => KE1	<p>Empirical Support of the MIE => KE1 is. xxx. Rationale: .</p>			
KE1 => KE2	<p>Empirical Support of the KE1 => KE2 is xxx. Rationale:</p>			
KE2 => KE3	<p>Empirical Support of the KE1 => KE2 is xxx. . Rationale:</p>			

1. Support for Biological Plausibility of KERs ₁	Defining Question	High	Moderate	Low
	<p>Is there a mechanistic (i.e., structural or functional) relationship between KE_{up} and KE_{down} consistent with established biological knowledge?</p>	<p>Extensive understanding based on extensive previous documentation and broad acceptance -Established mechanistic basis</p>	<p>The KER is plausible based on analogy to accepted biological relationships but scientific understanding is not completely established.</p>	<p>There is empirical support for a statistical association between KERs (See 3.), but the structural or functional relationship between them is not understood.</p>
MIE => KE1: (cut and paste the KER description into this cell)	<p>Biological Plausibility of the MIE => KE1 is xxx. Rationale:</p>			
KE1 => KE2: (cut and paste the KER description into this cell)	<p>Biological Plausibility of KE1 => KE2 is xxx Rationale:</p>			
KE2 => KE3 ((cut and paste the KER description into this cell)	<p>Biological Plausibility of KE1 => KE2 is xxx. Rationale:</p>			

2. Support for Essentiality of KERs ₅	Defining Question	High	Moderate	Low
	<p>What is the impact on downstream KERs and/or the AO if an upstream KER is modified or prevented?</p>	<p>Direct evidence from specifically designed experimental studies illustrating prevention or impact on downstream KERs and/or the AO if upstream KERs are blocked or modified</p>	<p>Indirect evidence that modification of one or more upstream KERs is associated with a corresponding (increase or decrease) in the magnitude or frequency of downstream KERs</p>	<p>No or contradictory experimental evidence of the essentiality of any of the KERs.</p>
AOP	<p>Rationale for Essentiality of KERs in the AOP is xxx:</p>			

Focus/Consistent Terminology – WOE for AOPs

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- Biological Plausibility – **KERs**
 - Biology of the pathway

- Essentiality – **KEs within AOP**
 - Necessity of Key Events
 - Experimental support normally from specialized studies to block or modify key events, stop/recovery studies

- Empirical Support – **KERs**
 - Pattern of Quantitative Associations among Key Events often considered through application of stressors

**More
important**

**Less
important**

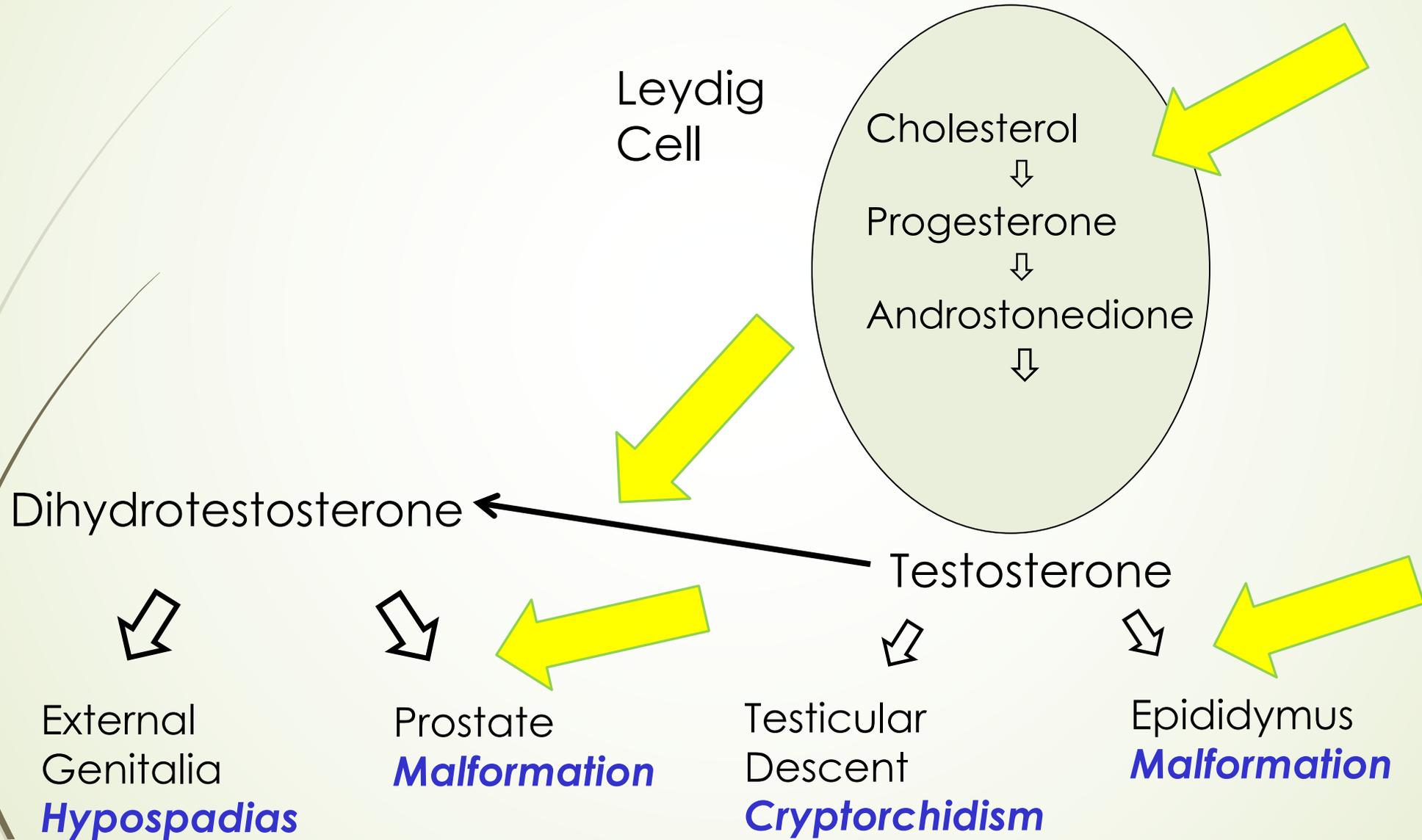


Biological Plausibility of KERs

- ▶ Strength of our hypothesis about **normal biology**, (structural/functional relationships)
 - ▶ The extent to which the relationships in a pathway are known, documented and accepted
 - ▶ Potential Measures?
- ▶ The extent to which we understand the pathway
 - ▶ Enables “prediction” or “testing” of the impact of disturbing it

Biological Plausibility

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Focus/Consistent Terminology – WOE for AOPs

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**More
important**

**Less
important**



Assembling Evidence - Essentiality of KEs

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- ▶ What is the impact on downstream KEs and/or the AO if an upstream KE is modified or prevented?
 - ▶ KEs are **necessary** elements of an AOP
- ▶ Directly measured experimental support (**direct evidence**) is most influential
 - ▶ e.g., **knockout models** – absence/reduction of KE_{down} when KE_{up} is blocked or diminished
 - ▶ e.g., **reversibility studies** where there is recovery when exposure is discontinued
 - ▶ i.e., blocking or reversing downstream responses by inhibiting (or allowing recovery) of upstream KEs

Essentiality

Assembling the Evidence

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Event	Direct Evidence	Indirect Evidence	No or contradictory experimental evidence	
			None	Contradictory
MIE				
KE1				
KE2				
KE3..... KE _n				

Weight of Evidence "Call"

Based on the supporting evidence for all KEs and the considerations in Annex 1, the weight of **evidence for the KEs in the context of the AOP overall** is:

High,

Moderate or

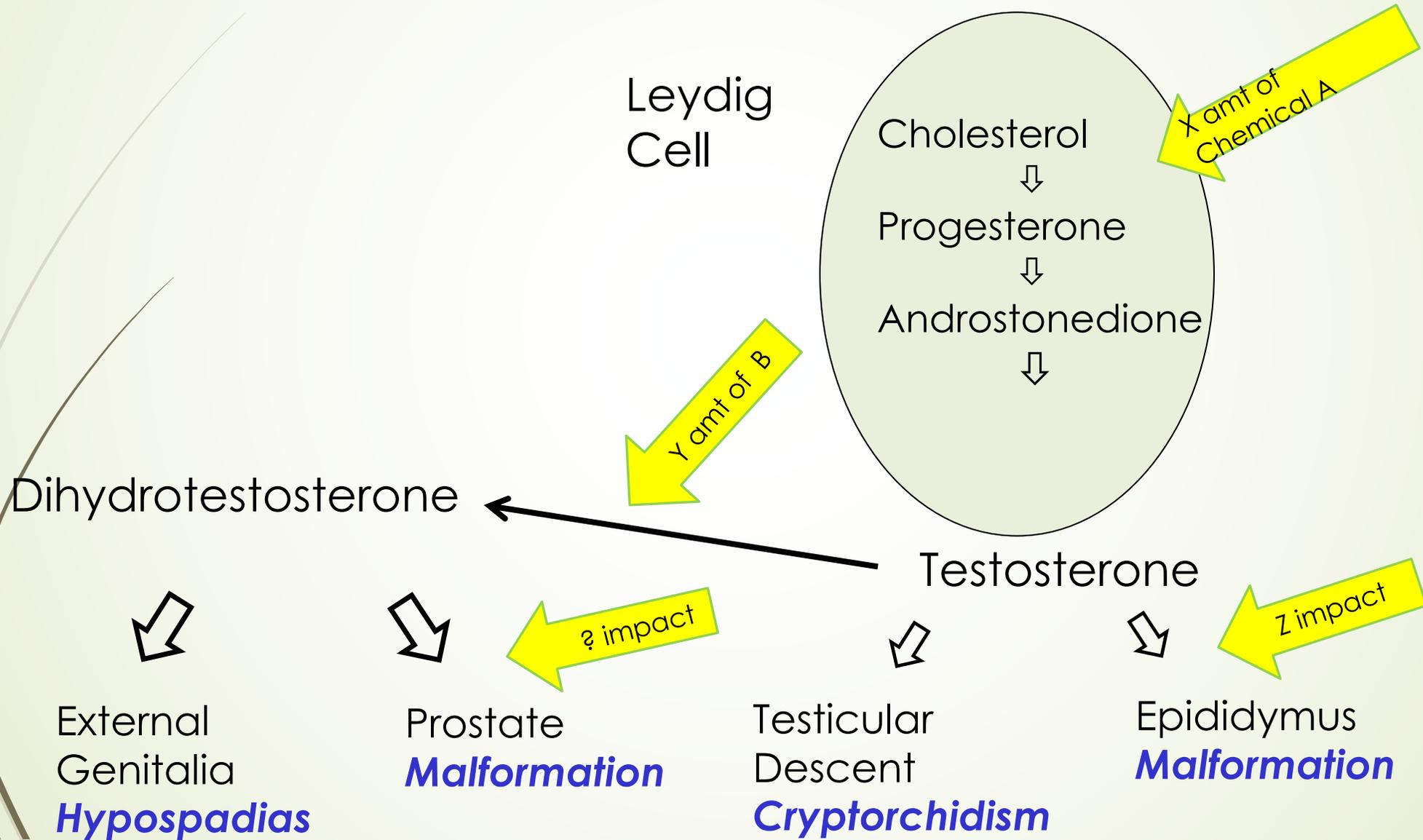
Low

Empirical Support

- Quantitative information on extent of the impact if some aspect of (a known or suspected) pathway is **perturbed** by a stressor
- Adding quantitative experimental support **for association** between key events to what we know about the biology
- Associations are often **tested** experimentally by application of various stressors

Empirical Support

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Empirical Support

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- ▶ Less influential than biological plausibility
 - ▶ Ranked below other considerations
 - ▶ Correlation \neq causation
- Rather, contributes in combination with biological plausibility
 - In general, if have strong biological plausibility, a small amount of empirical support can provide strong confidence.
 - If weak plausibility (structural/functional relationship not understood) – need a lot of empirical support to have predictive confidence

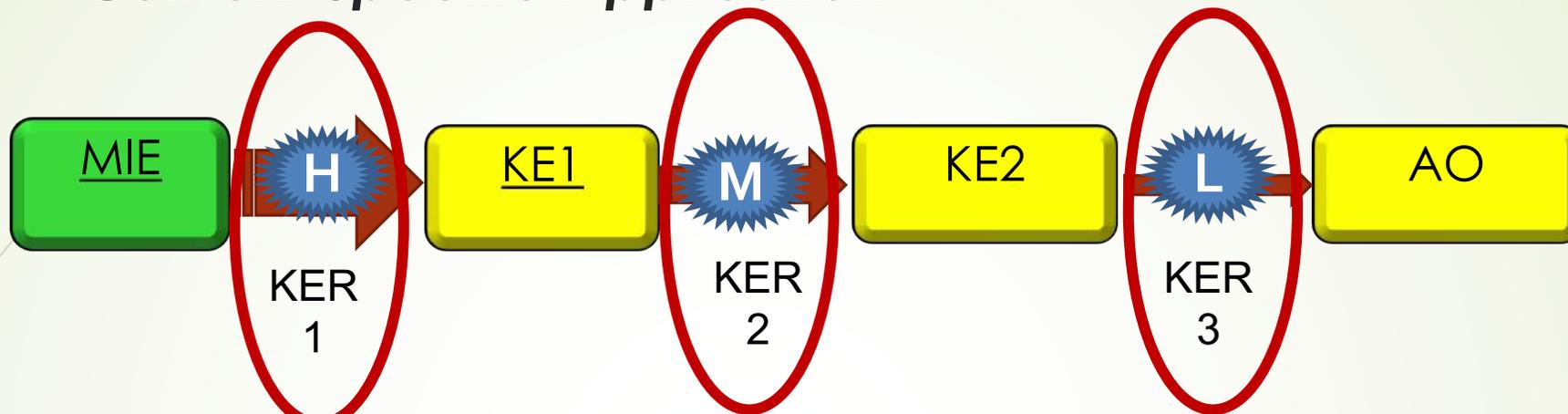
Concordance Tables For AOPs

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Chemical A and B thought to act on same MIE

Species	Chem	Conc.	KE1	KE2	KE3	KE4
FHM	A	1				
FHM	A	10				
FHM	A	100				
FHM	B	0.01				
FHM	B	0.1				
FHM	B	1				
RBT	B	0.05				
RBT	B	0.5				
RBT	B	2.5				
RBT	B	25				
RBT	B	250				

A "Snapshot/Network View" to Facilitate Consideration of Context Specific Application



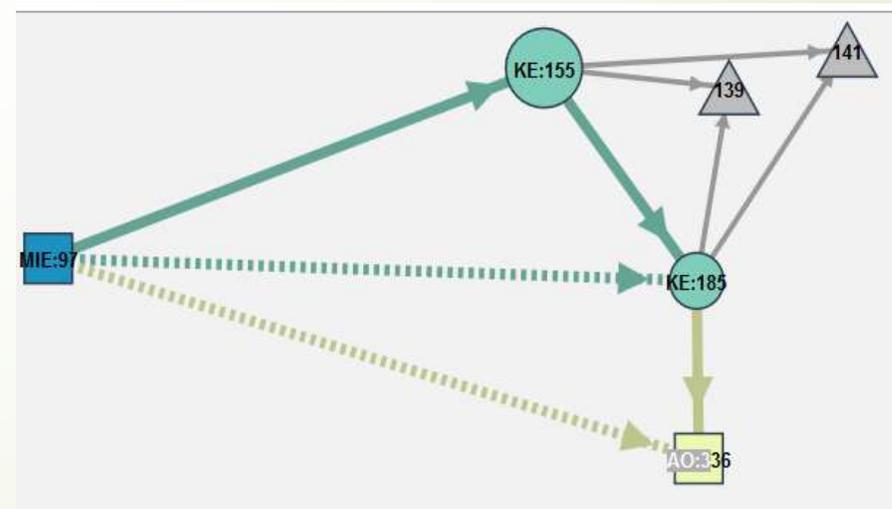
Confidence (Qualitative) Elements:

KERs – Biological Plausibility, Empirical Support (size of the arrow to represent H, M, L confidence)

Essentiality of KEs:

Event	Direct Evidence	Indirect Evidence	No or contradictory experimental evidence
MIE			
KE1			
KE2			
KE3.....			
KE _n			

Degree of Quantitation of KERs
Effectopedia



Best Practice - Weight of Evidence/Confidence Analysis

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- Distinguishing data supporting the various modified B/H considerations
- Characterizing nature of support for each of these considerations based on defining questions
- **Identifying inconsistencies/uncertainties** in supporting data
 - Templates/tables help
- Delineating consistent rationales for high, moderate and low confidence based on examples
- Identifying critical data gaps relevant to increasing confidence for regulatory application

References

Weight of Evidence

Meek et al. (2014a) New developments in the evolution and application of the WHO/IPCS framework on mode of action/species concordance analysis. *Journal of Applied Toxicology* **34**:1-18

Meek et al. (2014b) Mode of action human relevance (species concordance) framework: Evolution of the Bradford Hill considerations and comparative analysis of weight of evidence. *Journal of Applied Toxicology* **34**:595-606.

Guidance for AOPs

OECD (2014) Users' Handbook Supplement to the Guidance Document For Developing And Assessing AOPs

https://aopkb.org/common/AOP_Handbook.pdf

Examples

Becker et al. (2015) Increasing Scientific Confidence in Adverse Outcome Pathways: Application of Tailored Bradford-Hill Considerations for Evaluating Weight of Evidence. *Regul. Toxicol. Pharmacol.* **72**:514-537.

Yauk et al. (2015) Development of the adverse outcome pathway "alkylation of DNA in male premeiotic germ cells leading to heritable mutations" using the OECD's users' handbook supplement. *Environ. Mol. Mutagen* DOI 10.1002/em.21954

Expected Patterns for Empirical (Response-Response and Temporal) Support

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- ▶ Temporal Association (Time)
 - ▶ Early key events precede hypothesized late key events
- ▶ Response-Response (often considered on the basis of dose-response for applied stressors, as a surrogate)
 - ▶ The impact of early KEs is less than that for late KEs (severity↑)
 - ▶ Impact at increasing levels of biological organization to compromise normal function e.g., impact on cells vs. organs
 - ▶ Early key events occur at lower doses than late key events
 - ▶ For a given dose, the **incidence** (relative abundance/proportion impacted/frequency) of early key events is greater than or equal to that of later key events

e.g., reversible interaction with DNA → mutation → tumours