Evaluating AOP Evidence

Creating an Adverse Outcome Pathway in the AOP Wiki
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Presented by:
M.E. Meek, University of Ottawa
bmeek@uottawa.ca

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www.uOttawa.ca

Outline/Objectives

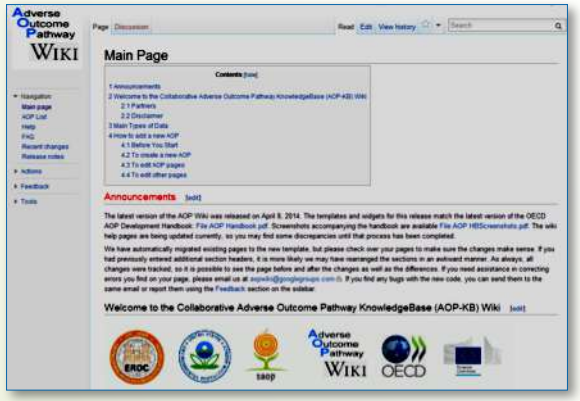
Why/How we evaluate evidence for AOPs

- 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

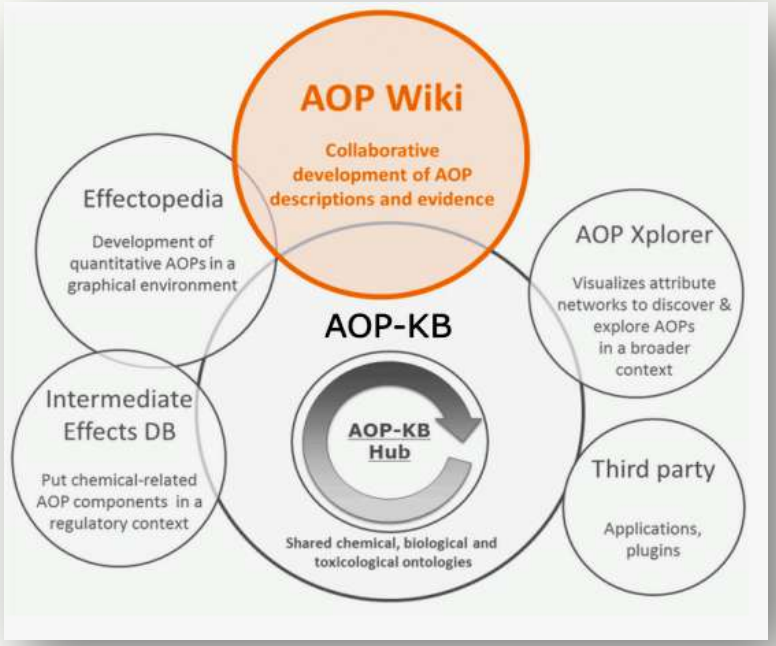
AOP Development and Description Case Studies

Users' handbook supplement to OECD guidance document for developing and assessing AOPs.

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

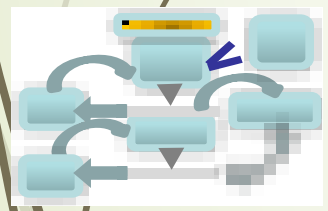
Tissue Dose → Key Event 1 Effective Dose → Key Event 2 → Key Event 3 → Adverse Outcome

Toxicokinetics (tk)

Toxicodynamics (td)

Chemical specific absorption, distribution, metabolism, excretion

Chemical agnostic biological pathway



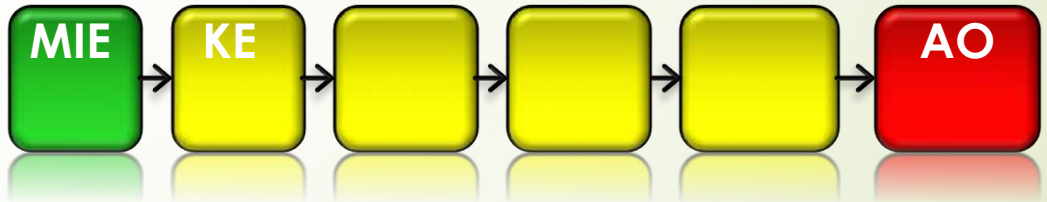
MOA Analysis; Biological Plausibility in Epi Studies

Integrated Testing

Monitoring of Environment

AOPs

Adverse Outcome Pathway (AOP)



KERs

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

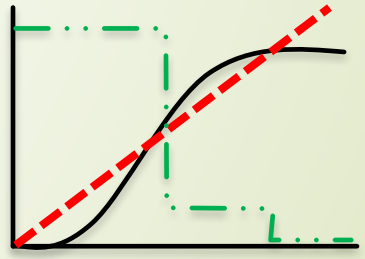
Weight of Evidence/Quantitation of KERs

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}?

AOP Wiki

Section 5b – MIE, KE, and AO descriptions

Annex 1

AOP Pa

Section 1 - Title
Section 2 - Authors
Section 3 – Status and Date Modified
Section 4A – Abstract
Section 4B – Background (optional)
Section 5A – Summary of the AOP
MIE
KEs
AO
Linkage table
Key Event Relationships/Associations
Applicability domain(s) of the AOP
Life-stage
Taxonomic
Sex
Section 7 – Assembly of Evidence - Essentiality
Section 8 – Overall Assessment of the AOP
Section 9 – Potential Applications of the AOP (Optional)

Consideration	Defining Questions	High	Moderate	Low
Biological Plausibility of KERs (S. 6)				
Support for Essentiality of KEs (S.7)				
Empirical Support for KERs (S.6.)				

MIE Page

Chemical initiator(s)

- Description
- Measurement/ detection
- Taxonomic applicability
- *Evidence for chemical initiation*

AO Page

- Description
- Measurement/ detection
- Taxonomic applicability
- *Regulatory relevance*

Section 6 – KER descriptions

KER Pages (section 6)

- Title
- Biological plausibility
- Empirical support
- Quantitative understanding
- Uncertainties and inconsistencies

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

Annex I

9

3. Empirical Support[b] for KERs	Defining Question	High	Moderate	Low
	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. Are there inconsistencies in empirical support across taxa, species and stressors that don't align with expected pattern for hypothesized AOP?	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	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..	**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**
MIE => KE1	**Empirical Support of the MIE => KE1 is. xxx. Rationale:**			
KE1 => KE2	**Empirical Support of the KE1 => KE2 is xxx. Rationale:**			
KE2 => KE3	**Empirical Support of the KE1 => KE2 is xxx. . Rationale:**			

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

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

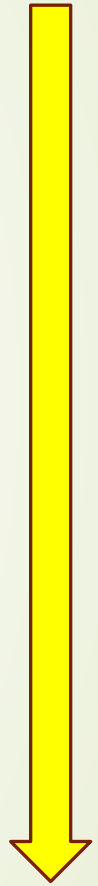
Focus/Consistent Terminology – WOE for AOPs

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

More important

- 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

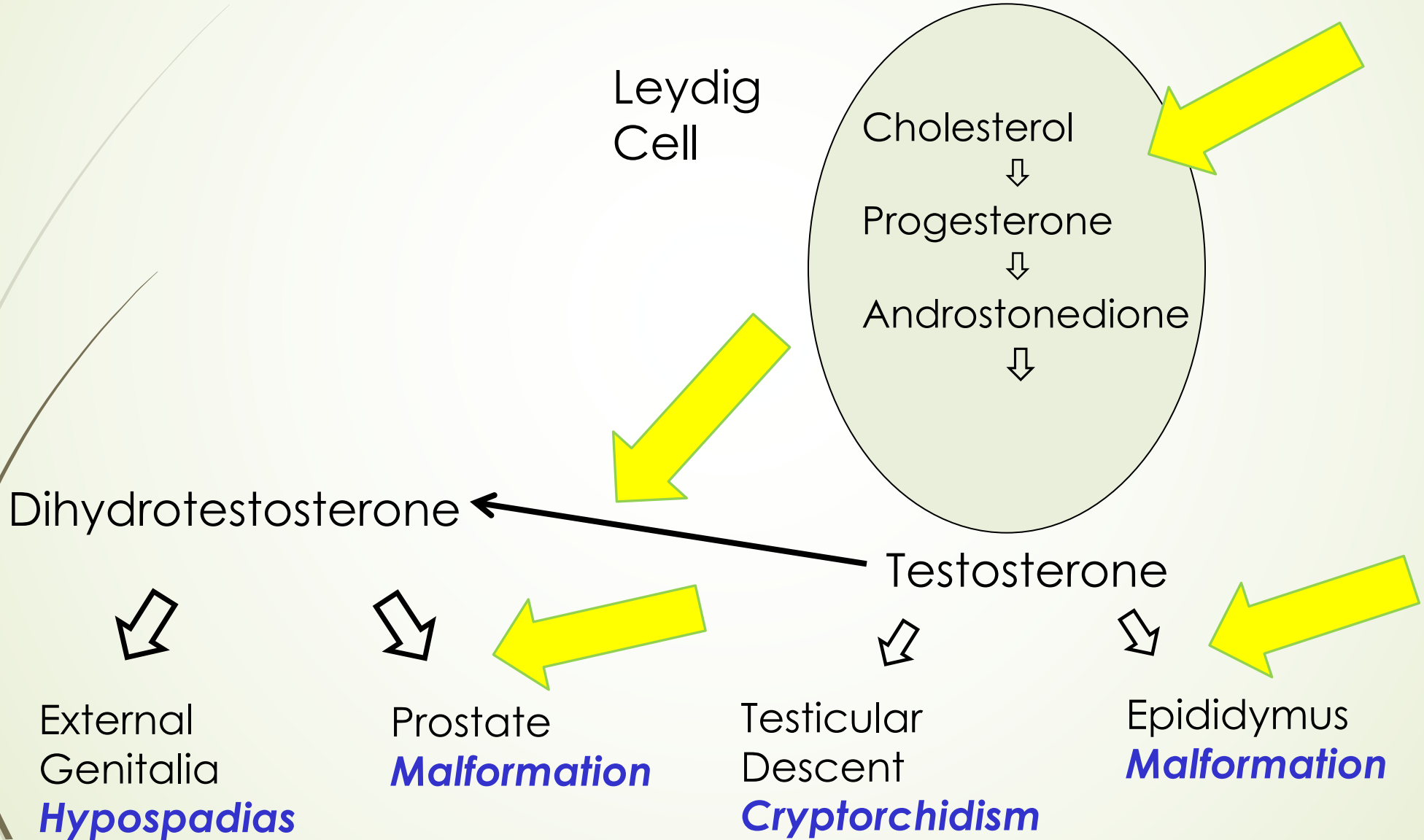


Less important

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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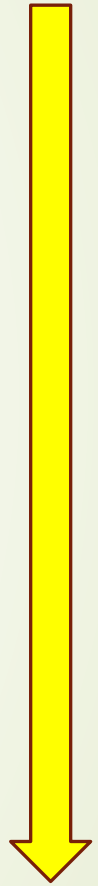


Less important

Assembling Evidence - Essentiality of KEs

- 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

Event	Direct Evidence	Indirect Evidence	No or contradictory experimental evidence	
			None	Contradictory
MIE				
KE1				
KE2				
KE3…… KEn				

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,

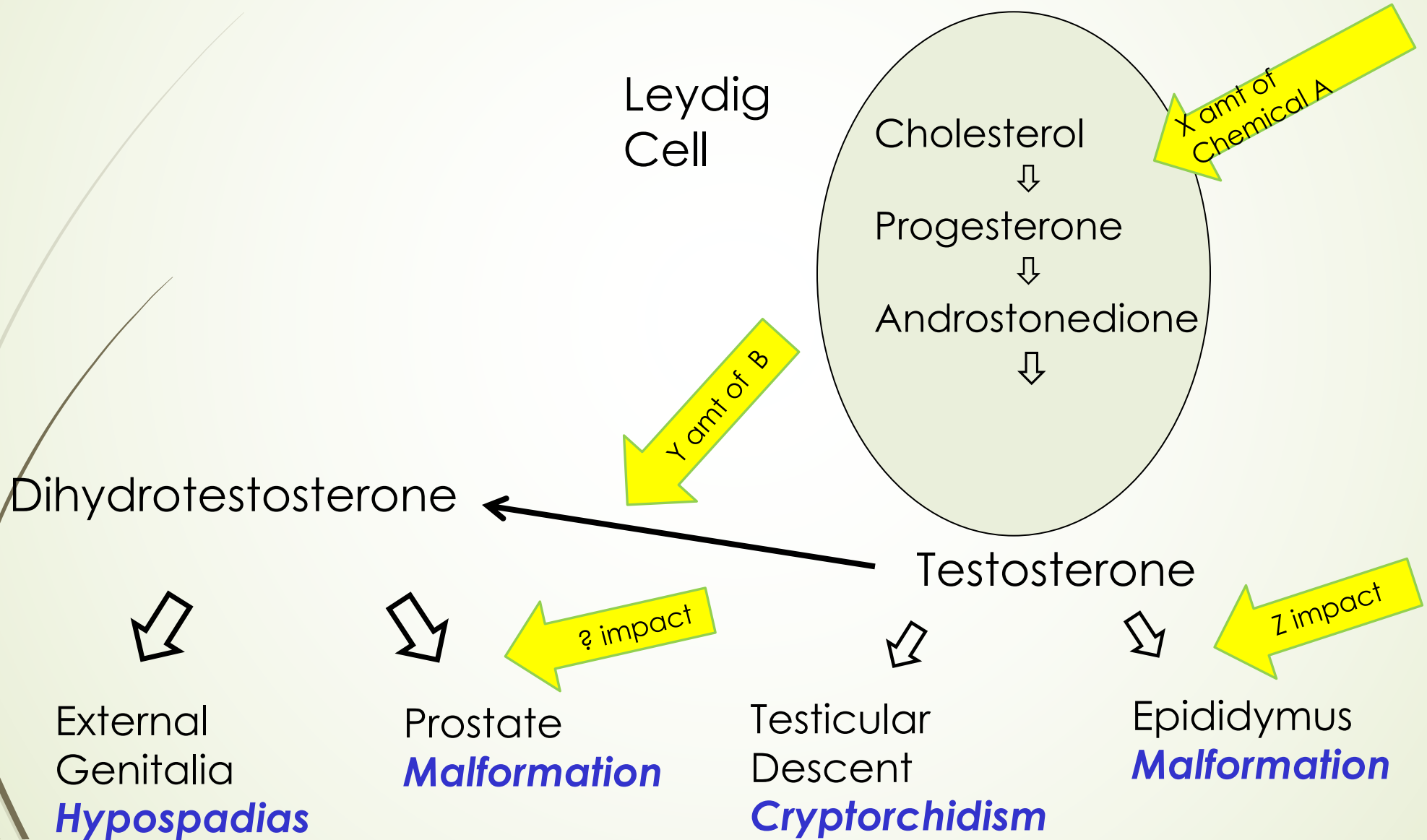
Moderate or

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



Empirical Support

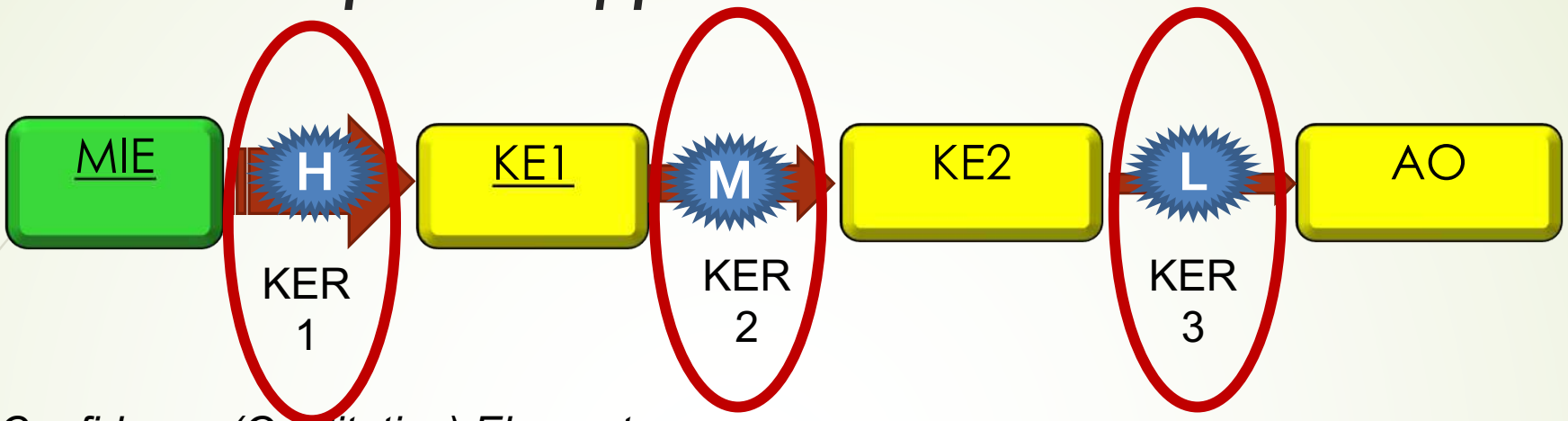
- Less influential than biological plausibility
  - Ranked below other considerations
    - Correlation ≠ causation
- Rather, contributes in combination with biological plausibility
  - In general, if have strong biological plausibility, a small amount of empirical support can provide strong confidence.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

Concordance Tables For AOPs

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



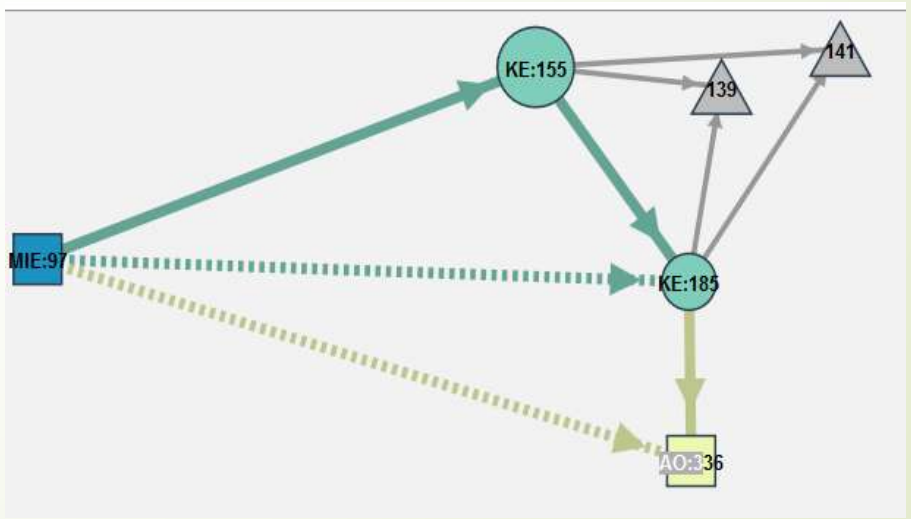
Confidence (Qualitative) Elements:

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

Degree of Quantitation of KERs
Effectopedia

Essentiality of KEs:

Event	Direct Evidence	Indirect Evidence	No or contradictory experimental evidence
MIE			
KE1			
KE2			
KE3…… KEn			



Best Practice - Weight of Evidence/Confidence Analysis

- 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

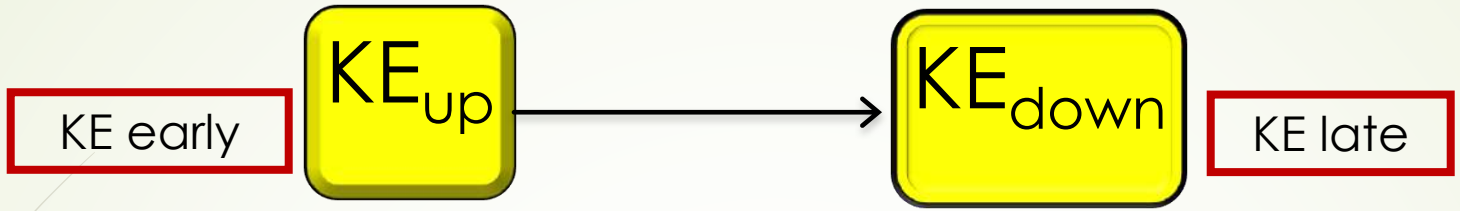
OECD (2014) Users' Handbook Supplement to the Guidance Document For Developing And Assessing AOPs https://aopkb.org/common/AOP_Handbook.pdf

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



- 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