ASSESSING THE COMPLETENESS OF ADVERSE OUTCOME PATHWAYS (AOPs)

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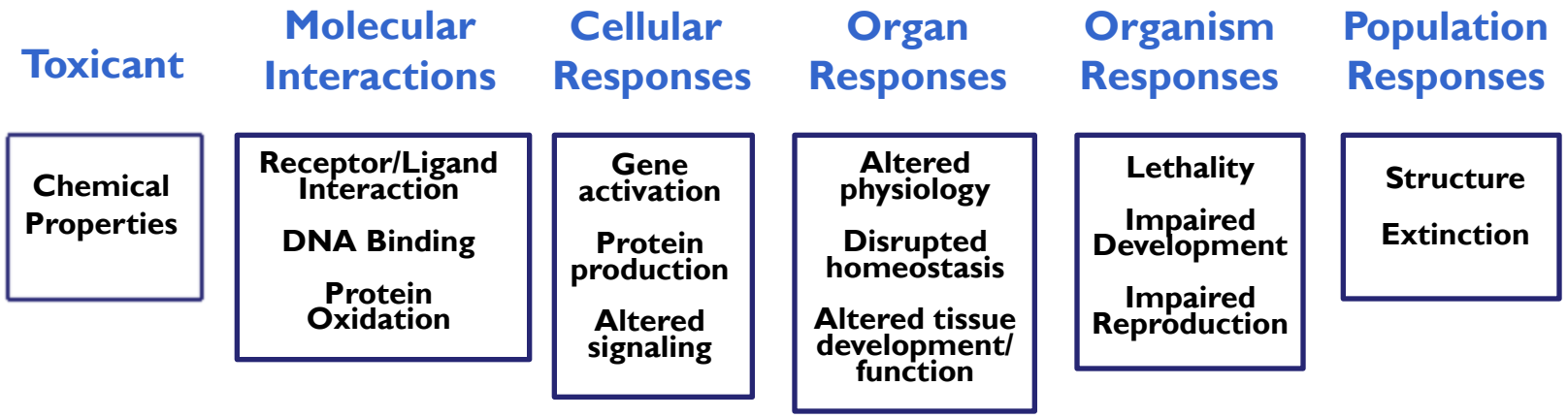
Background

AOPs delineate the documented, plausible, and testable processes by which a chemical induces molecular perturbations (Molecular Initiating Events) and the associated biological responses that describe how the molecular perturbations cause effects at the subcellular, cellular, tissue, organ, whole animal, and population levels of observation.

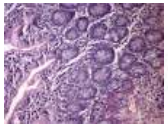


Terminology












Source | Environmental Containment | Exposure | Molecular Initiating Event | Organelle and Molecular Assemblies Effects | Cellular Effects | Tissue Effects | Organ Effects | Organ Systems Effects | Individual Effects | Population Effects | Community Effects

Toxicity Pathway

Mode of Action

Adverse Outcome Pathway

Source to Outcome Pathway

Adapted from K. Crofton 2010, OECD AOP Meeting Definitions

Seminal Features

Bottom-Up Approach- **e.g., *in chemico* to *in vitro* to *in vivo***

Plausible- **Believable and appearing likely to be true, often in the absence of direct proof (mechanistic understand).**

Testable- **Able to formulate hypotheses and test the hypotheses.**

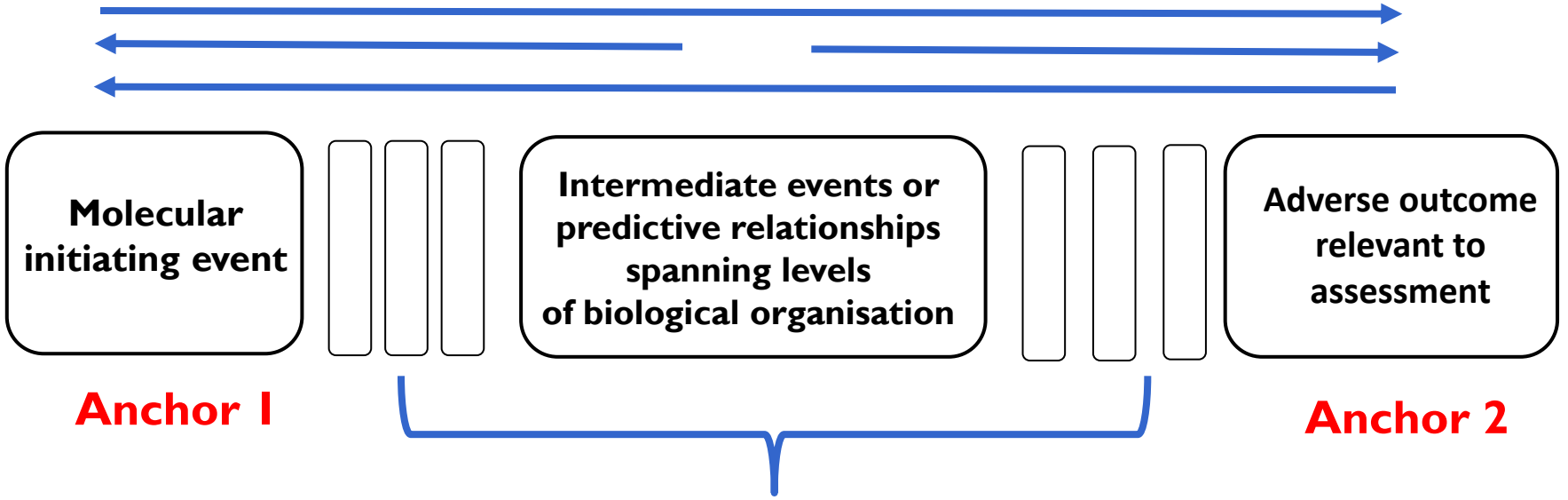
Uses of AOPs

The proposed uses of an AOP includes:

1. **A means of recording and formalising toxicity pathway information**
2. **Developing a Chemical Category**
3. **Developing Integrated Approaches to Testing and Assessment**
4. **Assisting in the Test Guideline Programme**

Development of an AOP

- the molecular initiating event (at the site of action)
- the intermediate steps and key events
- the apical adverse effect



Development of an AOP

The minimal requirements for information associated with the developed AOP:

- **Identification of the chemical-biological interaction - anchor 1**
- **Understanding of the apical outcome elicited by the MIE - anchor 2**
- **Identification of intermediate events depends on the level of knowledge about the outcome**

Key Events

are seminal intermediate events that are toxicologically relevant to the apical outcome.

are the basis for hypothesis development and testing. Thus, must be experimentally quantifiable.

Key Events

are typically based on rapid screening methods including protocols which assess *in chemico* and *in vitro* markers and signatures.

are likely to also include transcriptomics and proteomics, ex *vivo* results.

AOP Assessments

1. **Critical to be able to gauge the reliability and robustness of an AOP by evaluating the experimental support of the AOP.**

2. **The qualitative understanding of the AOP - assessment of the experimental evidence and empirical data; often based on a few well-studied compounds.**

3. **The quantitative understanding of the AOP - determining the response-to-response relationships required to scale *in vitro* effect to *in vivo* outcome. Not needed for category formation.**

4. **The assessment of the Weight-of-Evidence supporting the AOP by applying Bradford Hill criteria.**

Weight-of-Evidence Assessments

Decisions made with regard to the following criteria:

1. **Concordance of dose-response relationships,**
2. **Temporal concordance among the key events and adverse outcome,**
3. **Strength, consistency, and specificity of association of adverse outcome and initiating event,**
4. **Biological plausibility, coherence, and consistency of the experimental evidence,**
5. **Alternative mechanisms that logically present themselves and the extent to which they may distract from the postulated AOP. It should be noted that alternative mechanisms of action, if supported, require a separate AOP,**
6. **Uncertainties, inconsistencies and data gaps.**

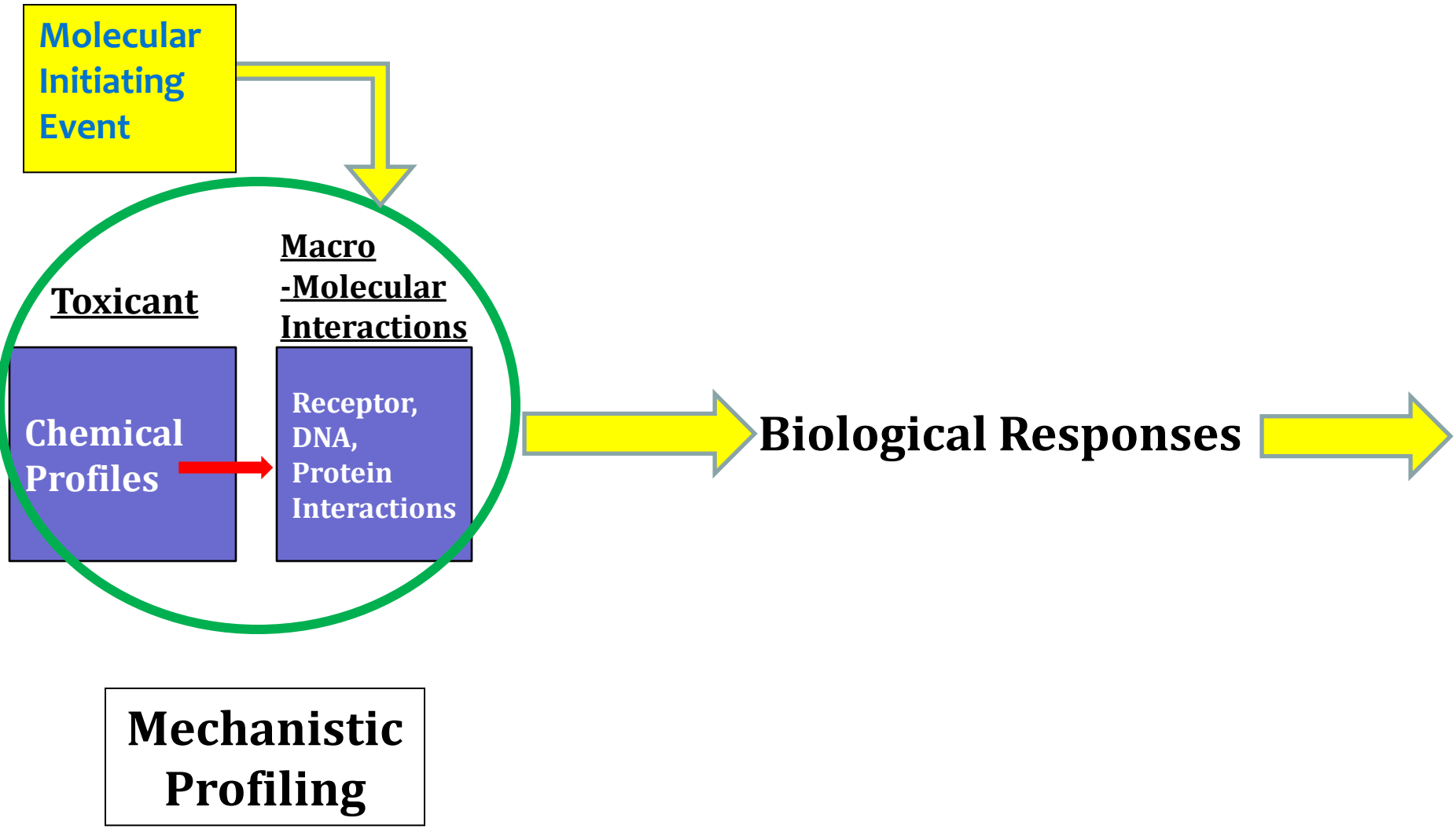
Best Principles for Developing an AOP

1. **An AOP should be based on a single, defined molecular initiating event and linked to a stated *in vivo* hazard outcome.**
2. **Assessment of the evidence in support of an AOP should include criteria based on the IPCS mode of action framework (Boobis et al., 2008).**
3. **Any framework used for AOP development should include a summary of the experimental support for the AOP, as well as a statement of:**
   A. **level of qualitative understanding of the AOP;**
   B. **consistency of the experimental data;**
   C. **confidence in the AOP;**
   D. **level of quantitative understanding of the AOP.**

Confidence in an AOP

Ascertained by Addressing the Following:

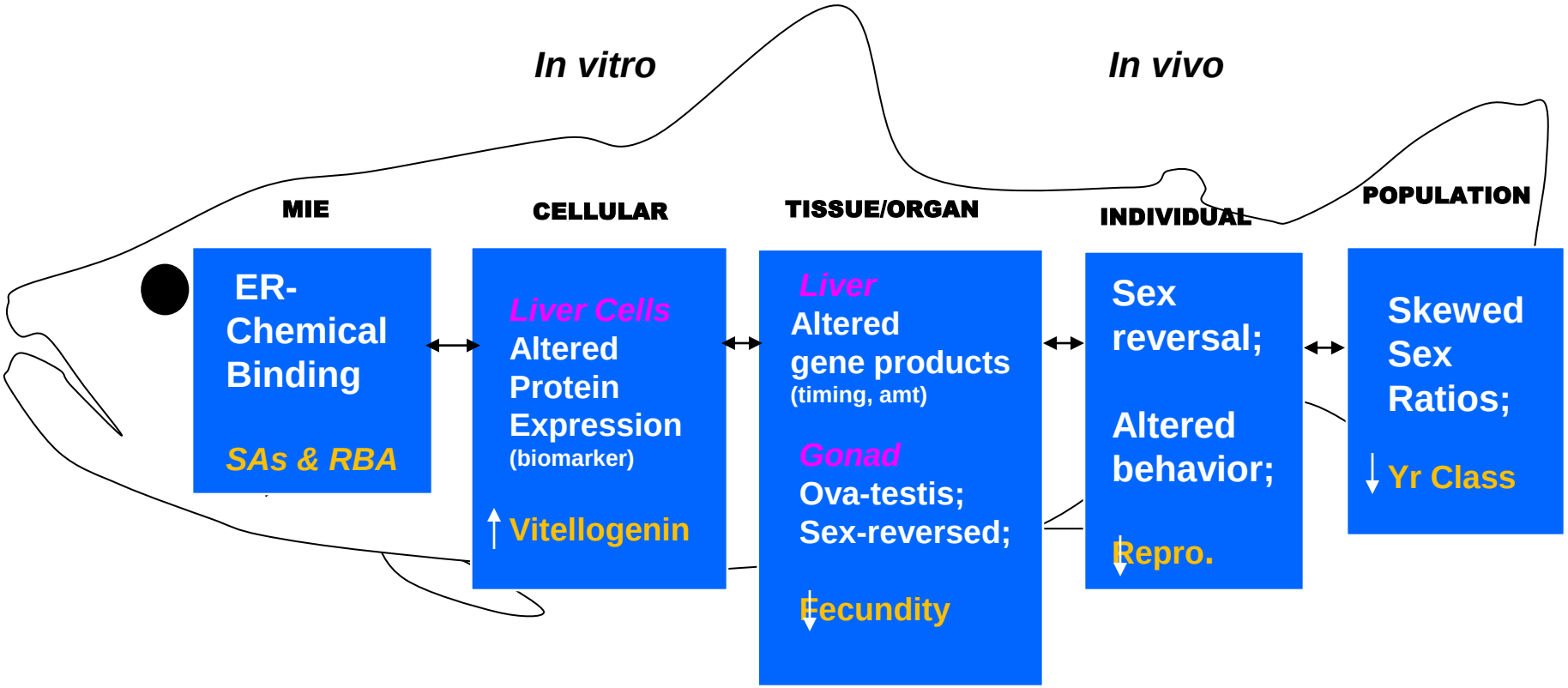
1. **How well characterized is the AOP?**
2. **How well are the initiating and other key events causally linked to the outcome?**
3. **What are the limitations in the evidence in support of the AOP?**
4. **Is the AOP specific to certain tissues, life stages / age classes?**
5. **Are the initiating and key events expected to be conserved across taxa?**



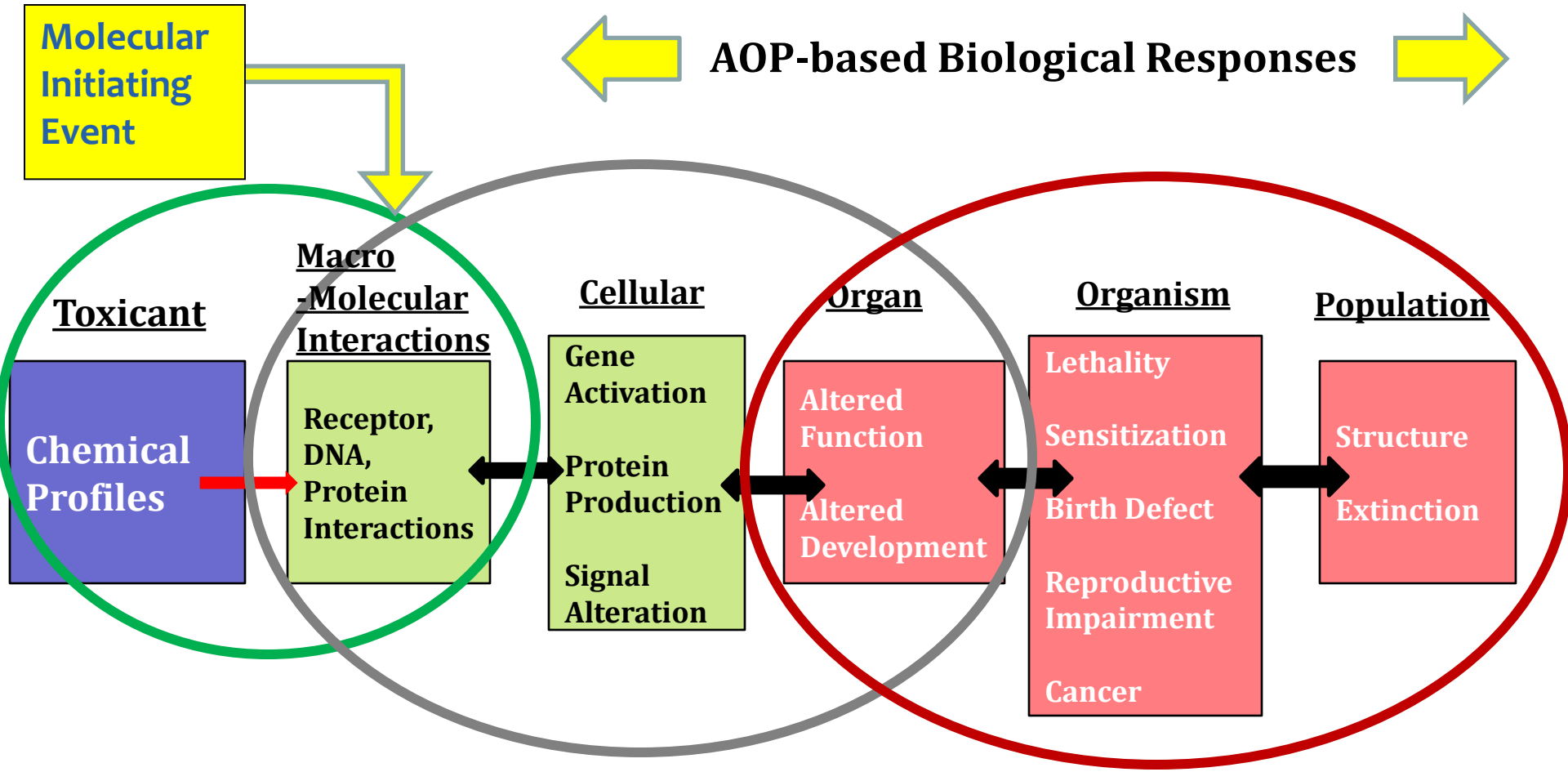
The Current Toolbox

AOP for ER-mediated Fish Reproductive Impairment

Measurements across levels of biological organization



Adapted from P. Schmieder 2009, OECD ER-binding AOP Meeting



Mechanistic Profiling

Cellular & In Vitro Sub-Profiling

In Vivo Sub-Profiling

The Future Toolbox

Thank You….

I will be happy to answer any questions.