WHAT IS THE ADVERSE OUTCOME PATHWAY (AOP) CONCEPT?

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Discussion Topics

Terminology

Variables that Control Toxicity

The Paradigm Shift

Adverse Outcome Pathway

Complexity in Toxicity

OECD AOP for Skin Sensitisation

Development & Assessments of an AOP

Best Principles

Terminology

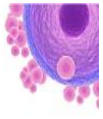

Source

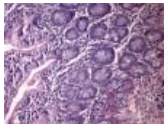

Environmental Containment


Exposure

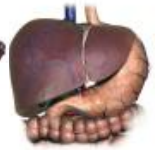

Molecular Initiating Event


Organelle and Molecular Assemblies Effects


Cellular Effects

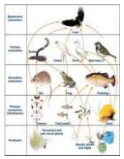

Tissue Effects


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

Variables that Control Toxicity

Two major variables.

The Molecular Initiating Event Matrix (MIEM)

A listing of relative binding affinities for all possible "biological receptors" that fall within the structural domain of a chemical.

The Response Matrix (RM)

A listing of the biological responses and coefficients between the different responses in all the subsystems (e.g. cells, tissues, organ etc.) and, ultimately, the *in vivo* endpoint of interest (e.g. skin sensitisation).

The Molecular Initiating Event Matrix

Chemical interactions are at the molecular level.

Most chemicals can interact with more than one molecular target.

Most chemicals have different affinities for different target.

The fasts reaction typically drives the *in vivo* toxicity.

The Response Matrix

Can be large, but it is finite.

Experience has shown that large portions can be scaled with a setting of 1.0.

Includes Molecular Screening and Toxicogenomics endpoints as well as traditional endpoints (e.g. histopathology).

The Paradigm Shift

Today we base chemical management largely on results from a battery of *in vivo* tests.

In the future we want to manage chemicals based on results from alternative methods (e.g. *in silico*, *in chemico*, and *in vitro* methods.

What Must We Do?

To move chemical management from an *in vivo* testing based process to an alternative methods based process:

Transparency,

Mechanistic plausibility.

And allow for hypothesis-based testing, especially with rapid and inexpensive screening methods.

How Do We Do This?

Integrating knowledge of the relevant chemicals interactions with biological systems (i.e. the molecular initiating events) with knowledge of the relevant biological responses or perturbations leading to the apical (e.g. *in vivo*) outcome of interest.

The Adverse Outcome Pathway

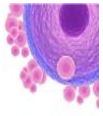

Source

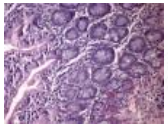

Environmental Containment


Exposure


Molecular Initiating Event


Organelle and Molecular Assemblies Effects


Cellular Effects

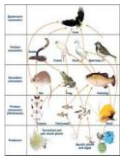

Tissue Effects


Organ Effects


Organ Systems Effects


Individual Effects

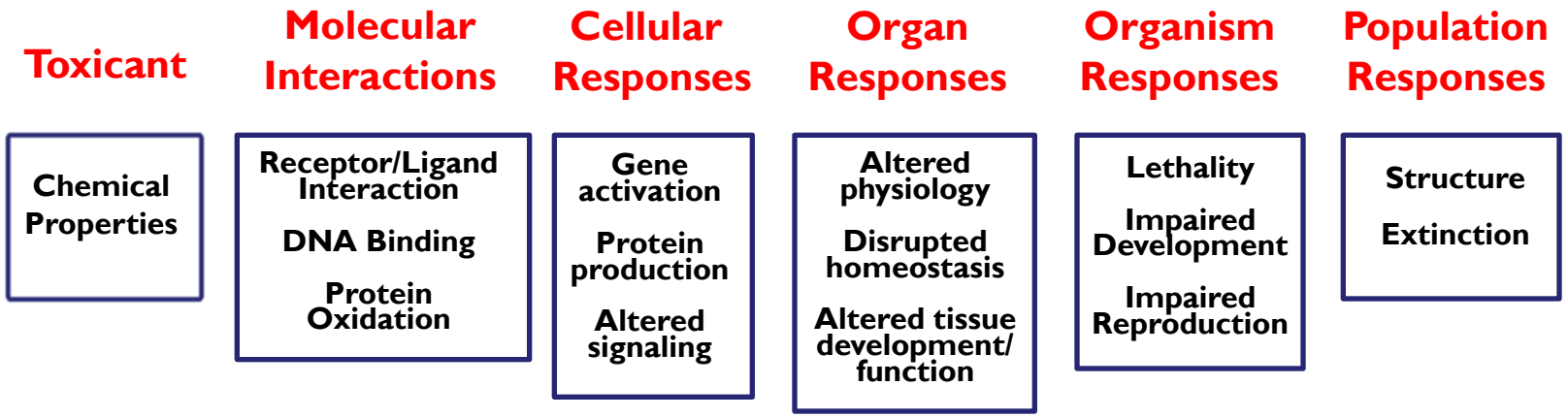

Population Effects


Community Effects

Adverse Outcome Pathway

Adverse Outcome Pathways

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.



Complexity in Toxicity

As the RM expands, it gives rise to the sense of complexity in toxicity.

This is especially the case for longer term health endpoints where effects are the result of multiple events (e.g. repeat dose toxicity), accumulates over time (e.g. neural toxicity) or are particular to a life stage of the organism (e.g. developmental toxicity).

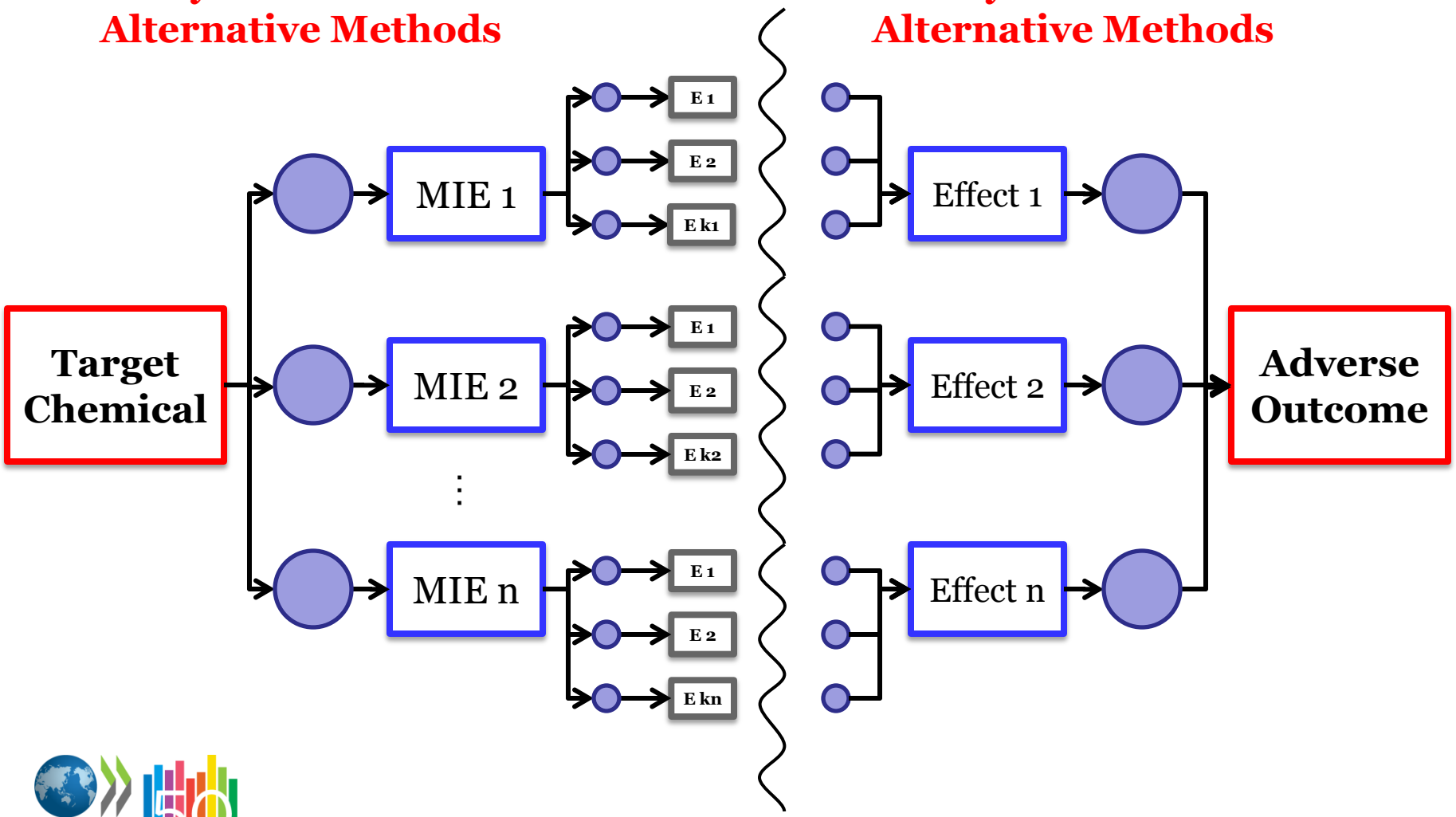
N-Dimensions of AOPs

Adapted from H. Aladjov 2012, OECD Effectopedia Meeting

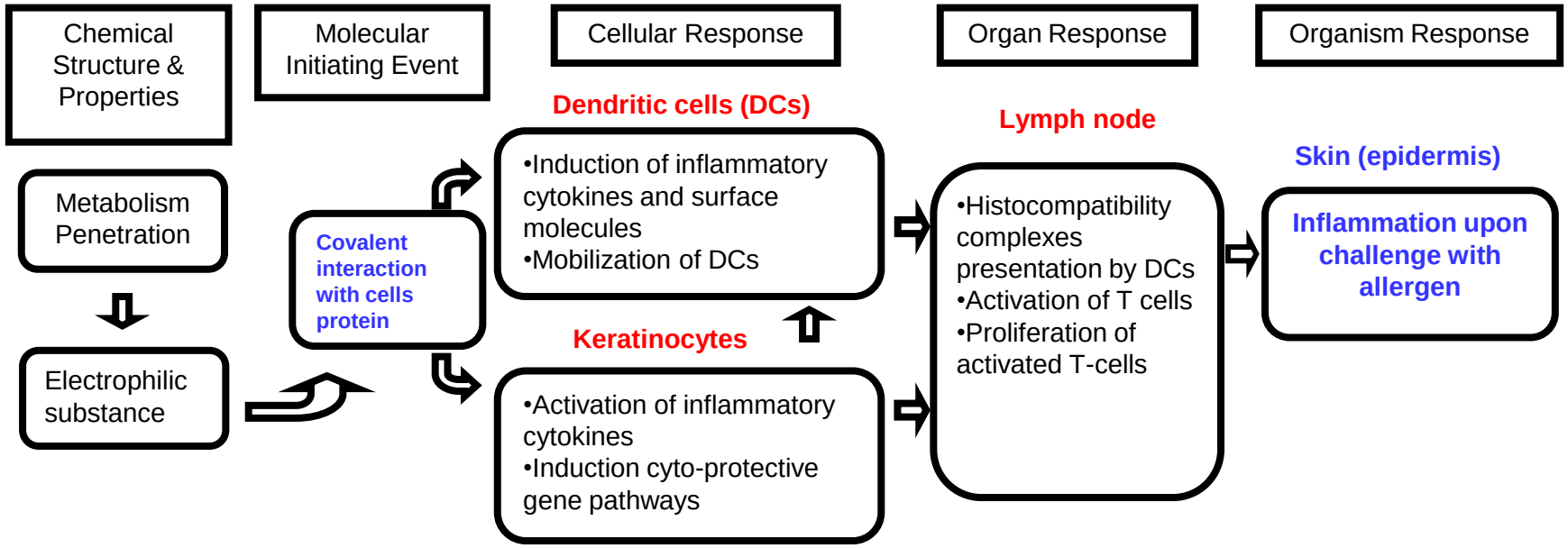
- Level of biological organization
- Gender
- Time to effect
- Taxonomy
- Life stage
- …

Handling Complexity

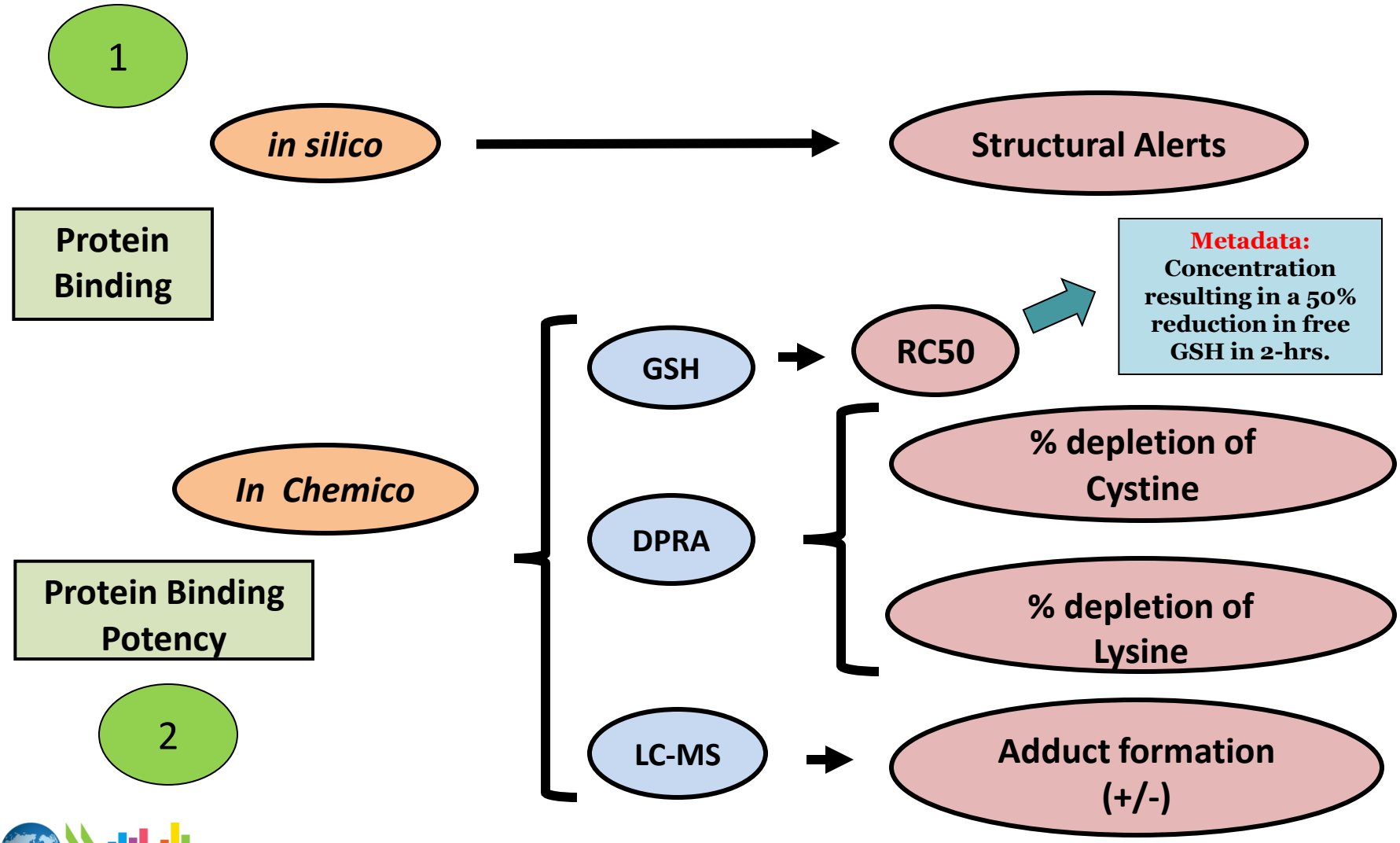
Adapted from H. Aladjov 2012, OECD Effectopedia Meeting

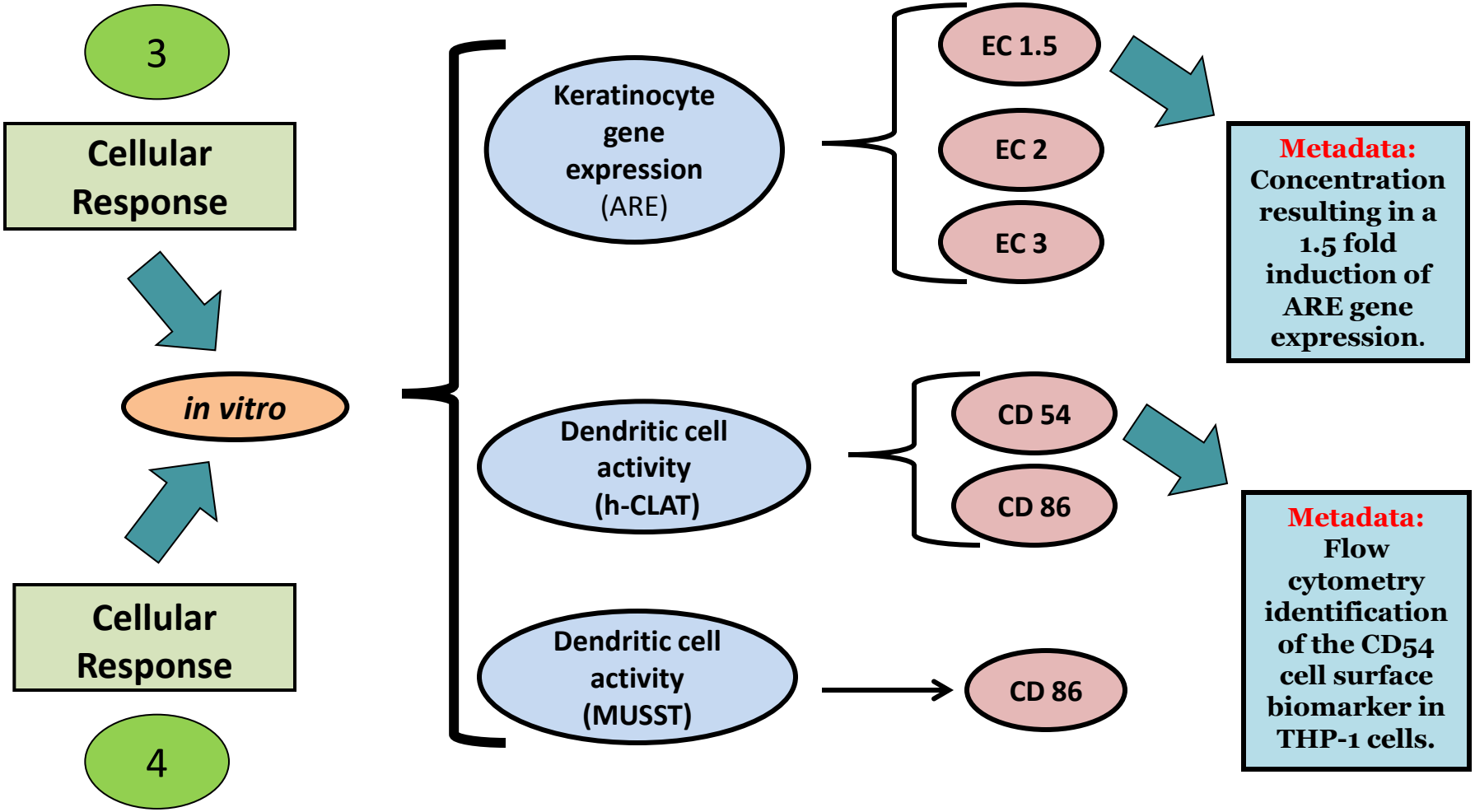


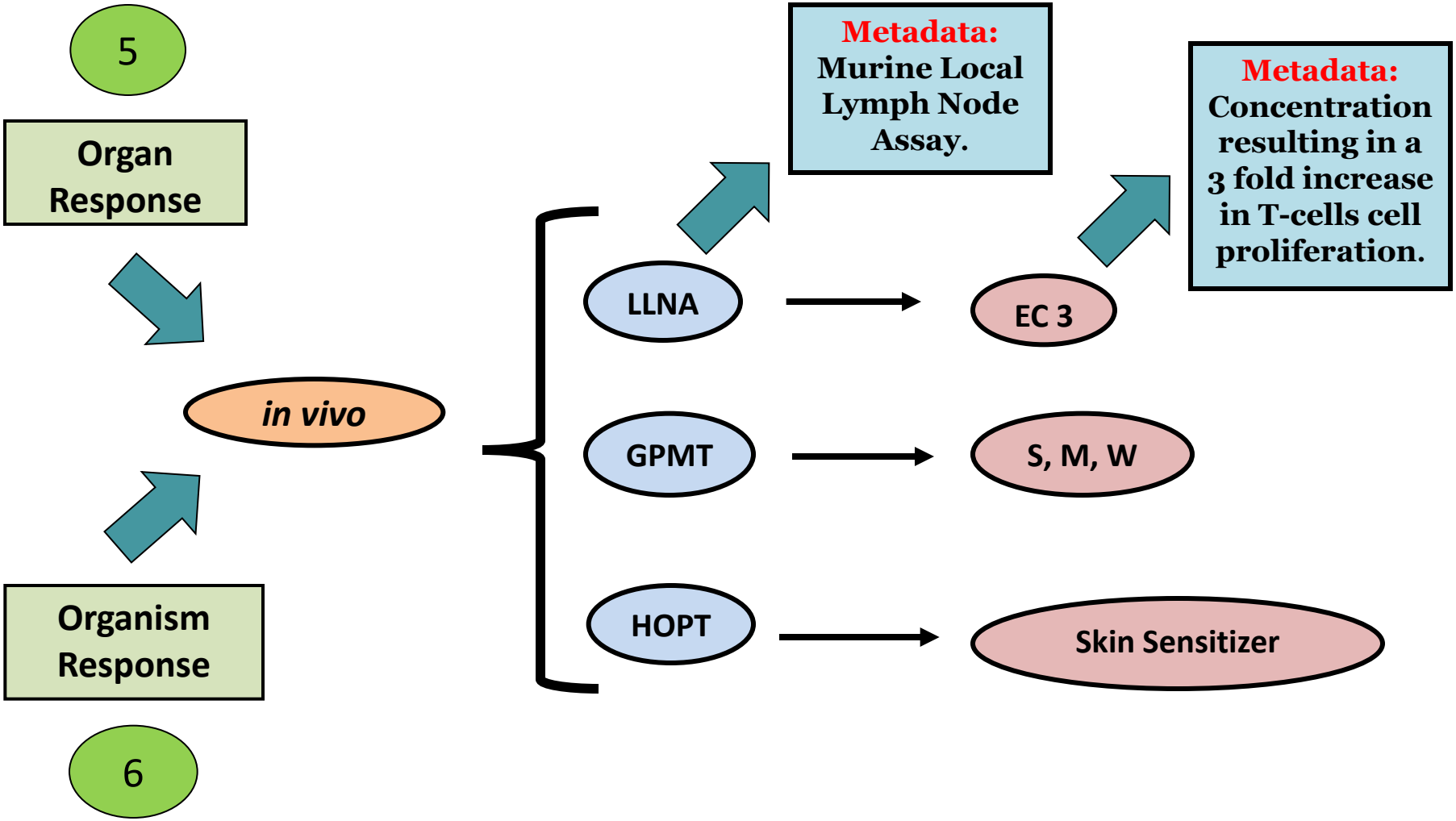
AOP for Skin Sensitisation

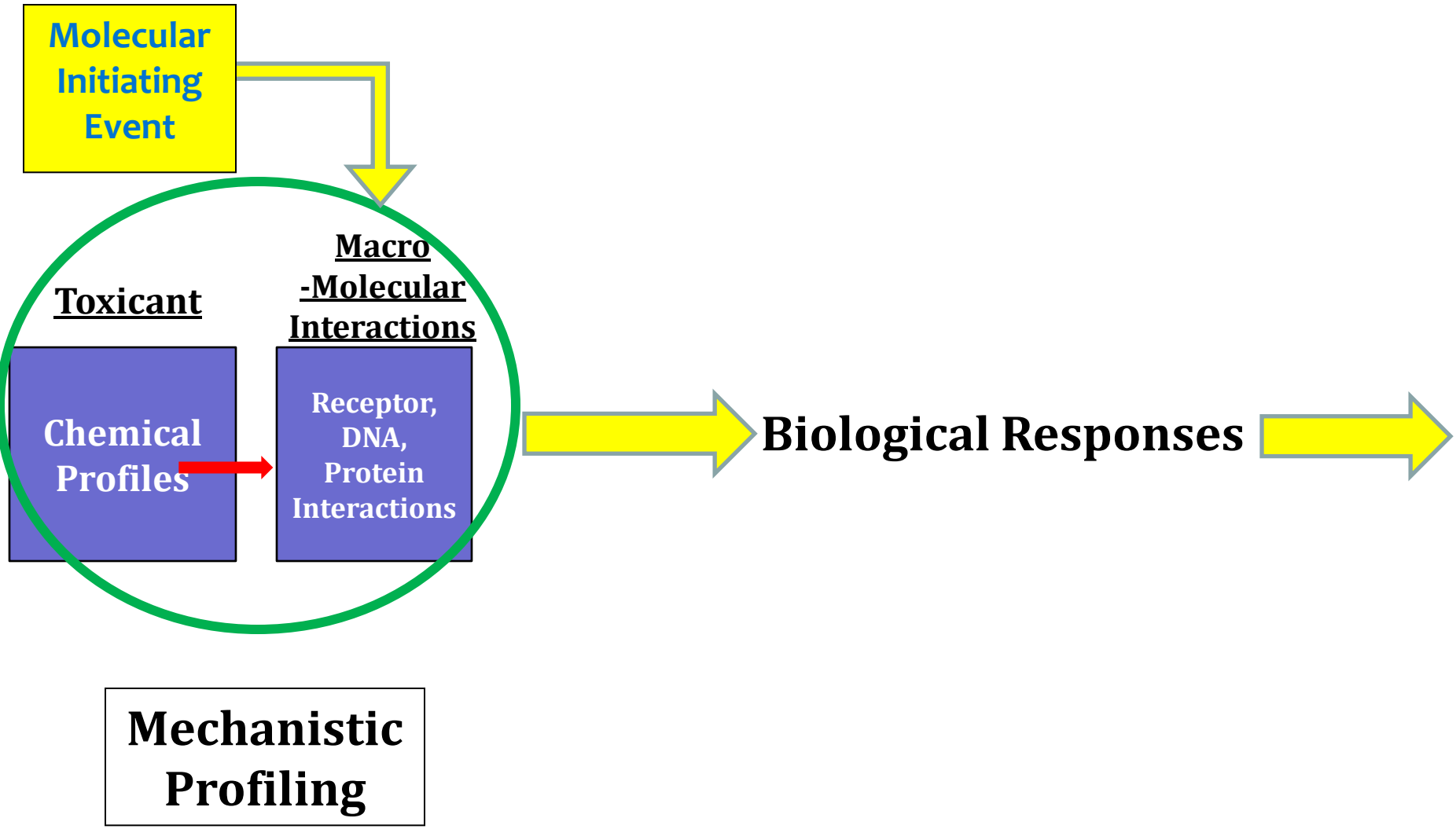


Adapted from The Adverse Outcome Pathway for Skin Sensitisation Initiated by Covalent Binding to Proteins. Part 1: Scientific Evidence
OECD ENV/JM/MONO(2012) 10 PART 1

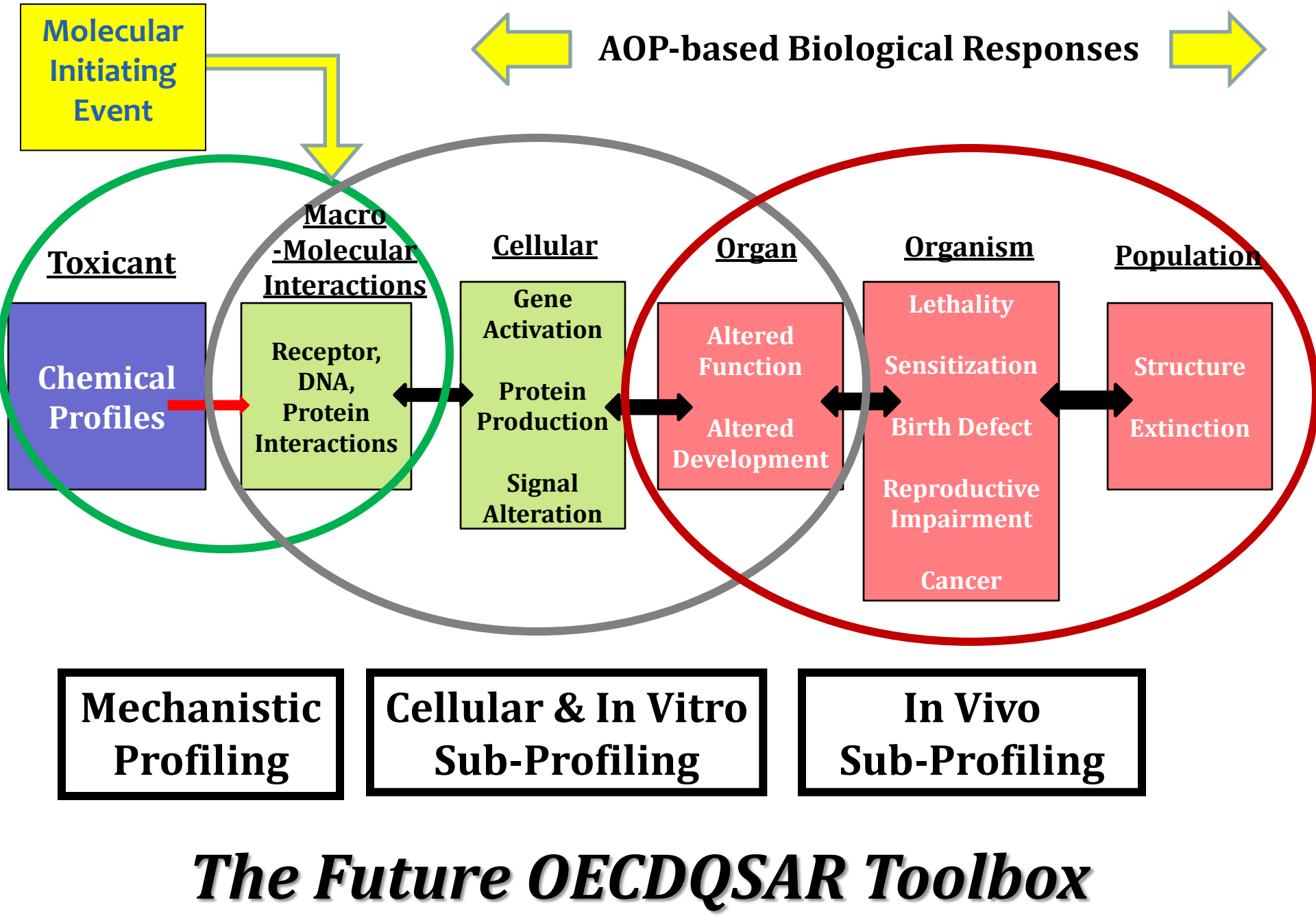

1
in silico
Structural Alerts
Protein Binding
Metadata: Concentration resulting in a 50% reduction in free GSH in 2-hrs.
GSH
RC50
In Chemico
% depletion of Cystine
DPRA
% depletion of Lysine
Protein Binding Potency
2
LC-MS
Adduct formation (+/-)


3
Cellular Response
in vitro
Cellular Response
4
Keratinocyte gene expression (ARE)
EC 1.5
EC 2
EC 3
Metadata: Concentration resulting in a 1.5 fold induction of ARE gene expression.
Dendritic cell activity (h-CLAT)
CD 54
CD 86
Metadata: Flow cytometry identification of the CD54 cell surface biomarker in THP-1 cells.
Dendritic cell activity (MUSST)
CD 86


5
Organ Response
in vivo
Organism Response
6
LLNA
GPMT
HOPT
Metadata: Murine Local Lymph Node Assay.
EC 3
Metadata: Concentration resulting in a 3 fold increase in T-cells cell proliferation.
S, M, W
Skin Sensitizer



The Current OECD QSAR Toolbox



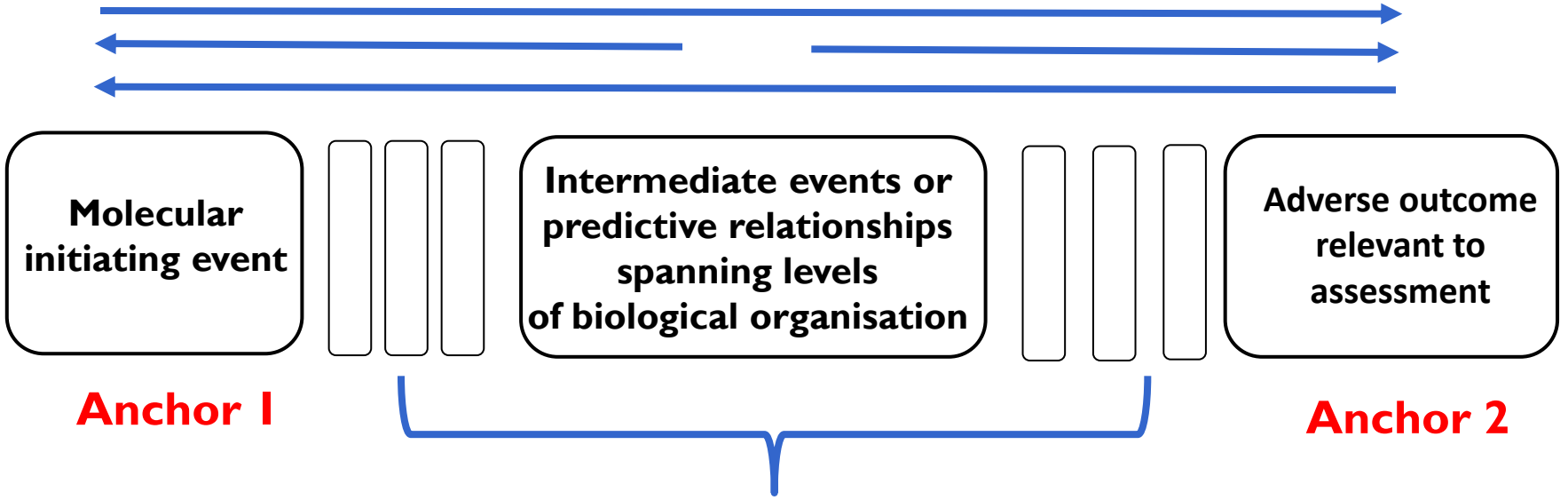
The Future OECDQSAR Toolbox

Development of an 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 this 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.

are often assessed by rapid screening methods.

AOP Assessments

Critical to be able to gauge the completeness (reliability & robustness) of an AOP by evaluating the experimental support of the AOP.

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

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

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.

Assessment of Completeness

Should include documented identification of:

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?

Assessment of Qualitative Understanding

Assessment should include documented identification of:

1) the molecular initiating event and molecular site of action (i.e. first anchor);

2) key cellular responses;

3) target tissue/organ(s) and key tissue or organ responses;

4) key organism responses; both physiological and anatomical;

5) (if required) key population responses;

6) Apical outcome of interest (i.e. second anchor).

Assessment of Weight-of-Evidence

Decisions made with regard to :

- **Concordance of dose-response relationships,**
- **Temporal concordance among the key events and adverse outcome,**
- **Strength, consistency, and specificity of association of adverse outcome and initiating event,**
- **Biological plausibility, coherence, and consistency of the experimental evidence,**
- **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,**
- **Uncertainties, inconsistencies and data gaps.**

Assessment of Quantitative Understanding

Should include quantification of:

1) the molecular initiating event;

2) other key events, especially cell-based ones;

3) development of response-to-response relationships required to scale *in vitro* effect(s) to *in vivo* outcomes.

Best Principles for an AOP

An AOP should:

be based on a single, defined molecular initiating event and linked to a stated *in vivo* hazard outcome.

include an evaluation of the experimental support for the AOP, to include a statement of:

1) Completeness of the AOP

2) Level of qualitative understanding of the AOP;

2) Consistency of the experimental data;

3) Confidence in the AOP based on Weight of Evidence;

4) Level of quantitative understanding of the AOP.

NEDO 28-day repeat dose toxicity project

The NEDO Hazard Evaluation Support System (HESS):

is consistent with the AOP approach .

is the first serious and credible attempt to deal with Repeat Dose Toxicity.

is a framework to be follows for other chronic endpoints.

Seminal Issue

Without a transparent description of a plausible progression of adverse effects at the different levels of biological organization etc., it is difficult to provide solid mechanistic reasoning for using alternative methods.

OCDE
DES POLITIQUES MEILLEURES
POUR UNE VIE MEILLEURE

The ideas presented here are those of the author, nothing noted should not be construed as official policy of OECD.

Thank You….