Applicability of AOPs in (regulatory) Hazard Assessment

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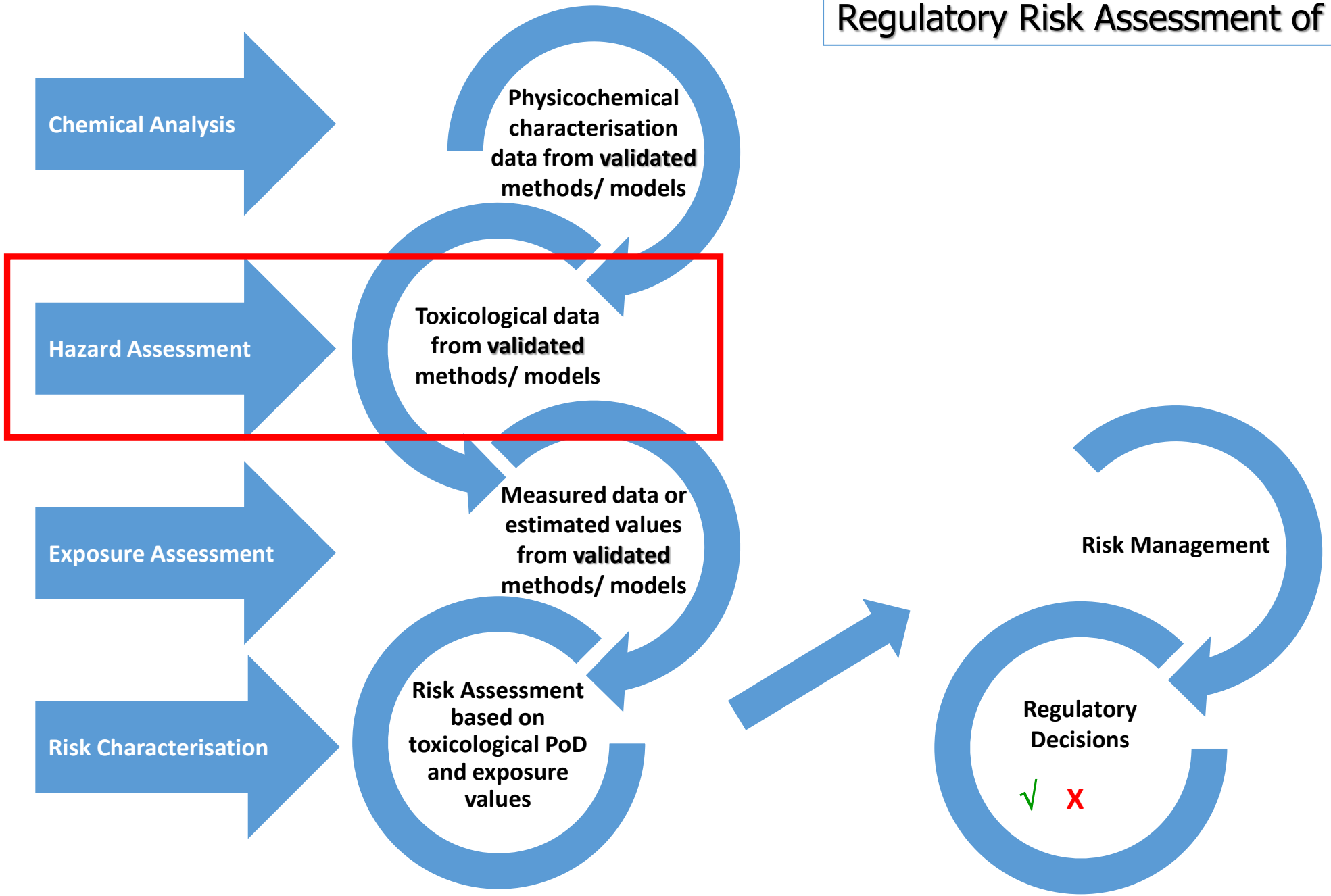
AOPs – Use in Regulatory Risk Assessments

- Brief overview of the mechanism of scientific advice for regulatory risk assessment of chemicals
- How AOPs can inform risk assessment process?
- What is the current perspective on the use of AOPs in regulatory risk assessments?

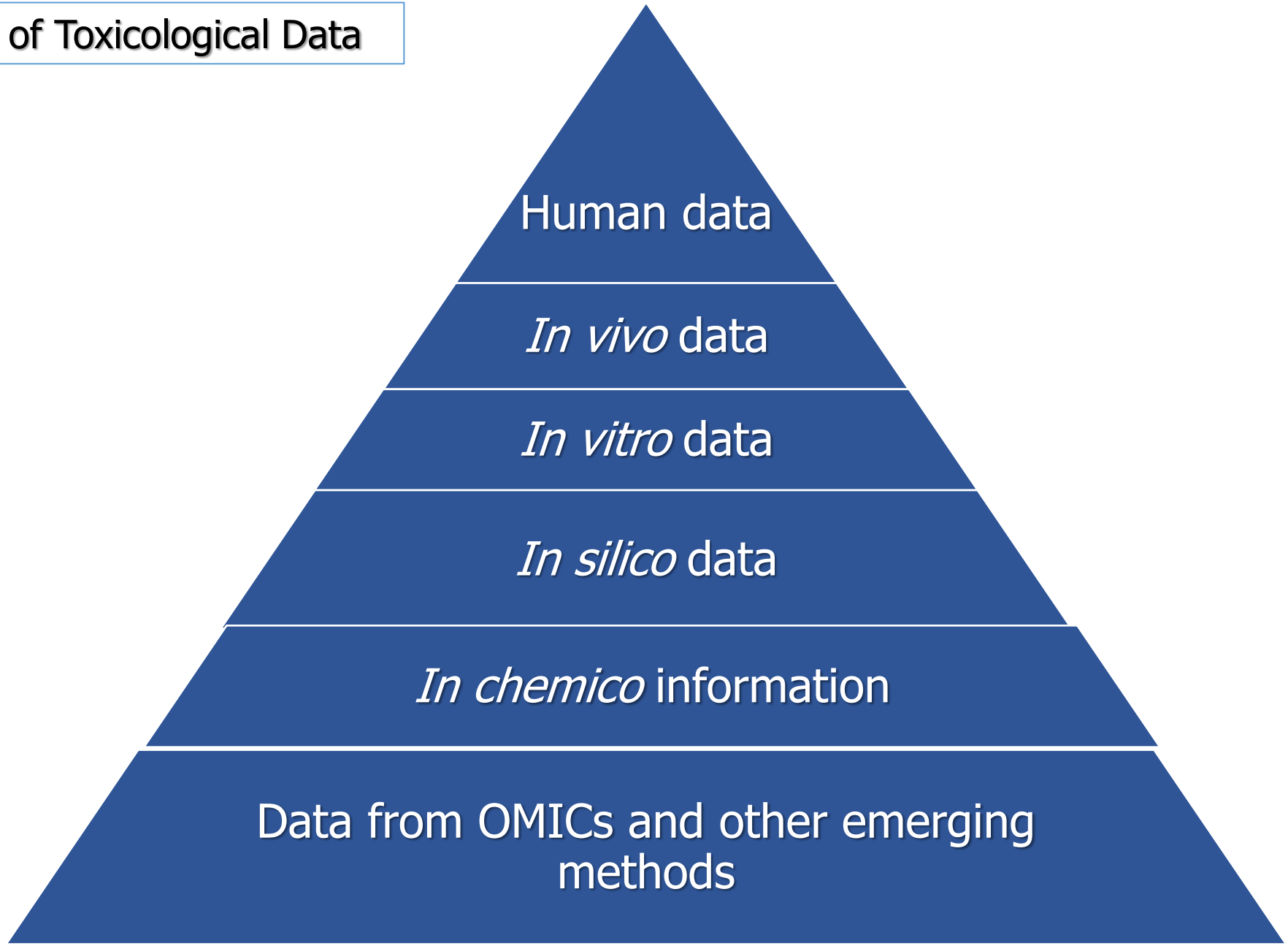
The EU Mechanism of Scientific Advice for Regulatory Risk Assessment of Chemicals

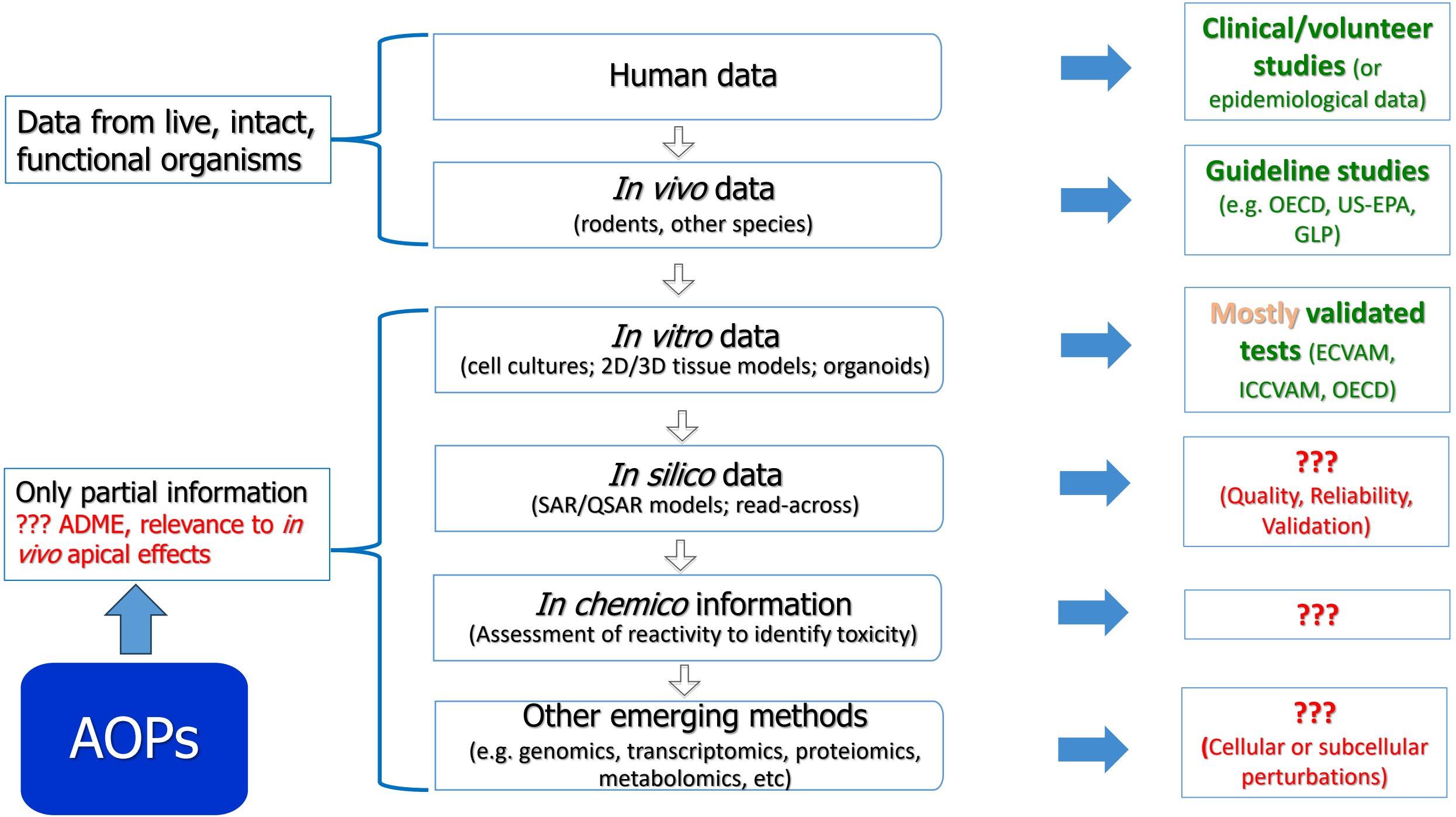


Moving Away from Animal Testing

- Legislative provisions are in place in all EU regulations for the Reduction, Refinement and Replacement (3Rs) of the use of test animals in laboratory procedures;
- Increasing emphasis on the use of NAMs – animal testing only as a last resort;
- Cosmetic Regulation (EC) 1223/2009 is the first one to have completely banned animal testing of cosmetic ingredients/ products, and marketing of cosmetic ingredients/ products tested on animals since 11 March 2013.

Hierarchy of Toxicological Data




Data from live, intact, functional organisms
Human data
In vivo data
(rodents, other species)
In vitro data
(cell cultures; 2D/3D tissue models; organoids)
In silico data
(SAR/QSAR models; read-across)
In chemico information
(Assessment of reactivity to identify toxicity)
Other emerging methods
(e.g. genomics, transcriptomics, proteiomics, metabolomics, etc)
Clinical/volunteer studies (or epidemiological data)
Guideline studies
(e.g. OECD, US-EPA, GLP)
Mostly validated tests (ECVAM, ICCVAM, OECD)
???
(Quality, Reliability, Validation)
???
???
(Cellular or subcellular perturbations)
Only partial information
??? ADME, relevance to in vivo apical effects
AOPs

NAMs: 'Officially-validated' and 'Scientifically-valid'

- Generally, data for regulatory risk assessments are only accepted from validated tests carried out in accordance with appropriate Guidelines,
- Most regulatory risk assessors also consider well documented scientifically-justified methods that may not have been officially validated yet, on a case-by-case basis;
- A single NAM is unlikely to provide sufficient evidence – a combination of NAMs is generally necessary to develop a weight of evidence (WoE) for use in risk assessment;
- The WoE increases if NAMs are aligned to address KEs in an AOP.

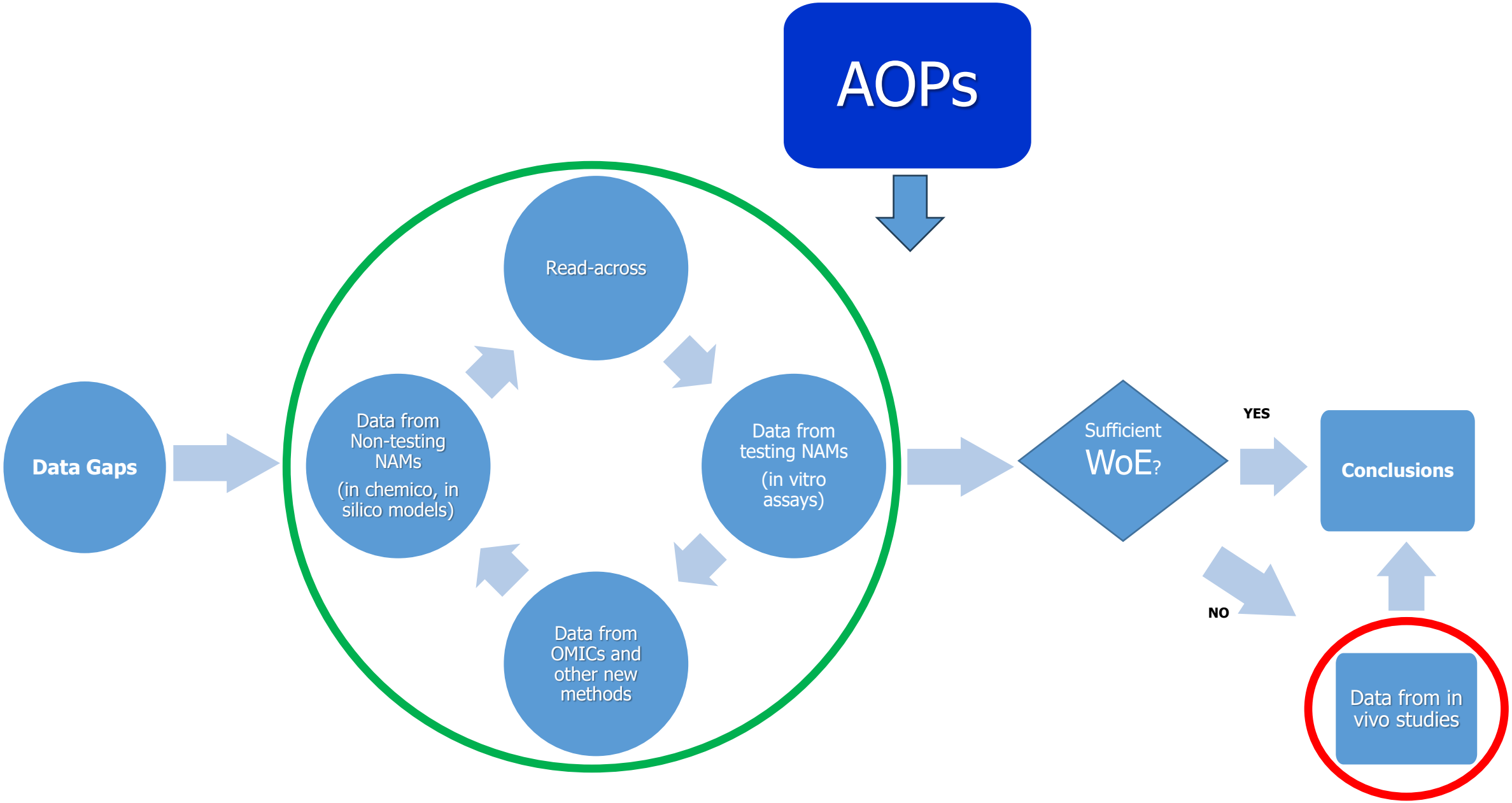
Building a Credible Picture from Pieces of the Evidence

Can NAMs data alone give a risk assessor the same level of confidence as the data from a traditional in vivo test?

- The answer seems to have gradually moved over the years from 'unlikely' to 'may be' to 'potentially' and 'yes' for some endpoints, such as skin irritation/ corrosion, skin sensitisation, phototoxicity, mutagenicity/ genotoxicity, endocrine activity, 'A' of ADME, and partially for acute toxicity and carcinogenicity.
- More complex endpoints are still a challenge, such as sub-chronic/chronic repeated dose toxicity, reproductive/developmental toxicity, non-genotoxic carcinogenicity, endocrine disruption.
- This is where aligning NAMs with AOPs can make them more useful for risk assessment.

Available NAMs

Toxicological endpoint	*In silico* models/ read-across	Validated *in vitro* tests
Acute Toxicity	☑	☑
Skin corrosion/irritation	☑	☑
Skin sensitisation	☑	☑
Phototoxicity	☑	☑
Toxicokinetics	☑	☑A ☒DME
Repeated dose toxicity/ chronic toxicity	☑	☒
Reproductive & developmental toxicity	☑	☒
Mutagenicity/genotoxicity	☑	☑
Carcinogenicity	☑	☒ CTA
Endocrine activity	☑	☑EA ☒ED


AOPs
Data Gaps
Read-across
Data from Non-testing NAMs (in chemico, in silico models)
Data from testing NAMs (in vitro assays)
Data from OMICs and other new methods
Sufficient WoE?
YES
NO
Conclusions
Data from in vivo studies

AOPs as a Supporting Evidence for Deriving Conclusions

- AOPs (like other evidence) can be used as supportive data to inform risk assessment;
- AOPs are already widely used for simpler endpoints, but their practical value for more complex ones is still uncertain/limited;
- The importance of AOPs in supporting the use of NAMs data in risk assessments is widely recognised across regulatory bodies/commitees;
- The development and implementation of AOP across multiple endpoints features heavily in identified research needs of ECHA*

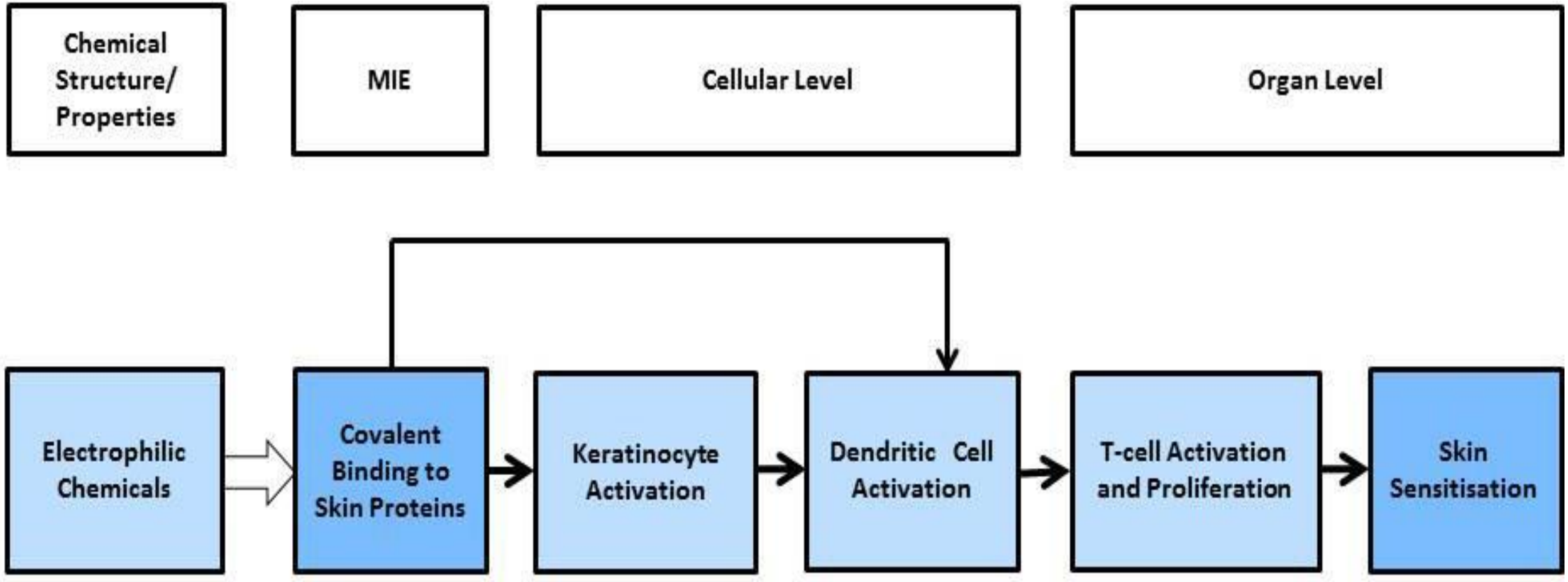
*https://echa.europa.eu/documents/10162/17228/key_areas_regulatory_challenge_en.pdf/fbaa76cf-acd0-0c8a-5dd7-3195379946aa

AOPs as a Standalone Evidence for Deriving Conclusions

- OECD endorsed AOPs can be used to demonstrate presence of the certain hazard;
- It is also possible to conclude on the lack of effect, if one or more AOPs cover sufficient toxicological space of the regulatory endpoint (e.g. SIR under REACH). This has so far been only demonstrated for a few less complex endpoints (e.g. skin sensitisation);
- The use of 3 (in vitro) KEs for skin sensitisation is embedded into REACH information requirements and Guidance*. Work under GHS is also ongoing to introduce the in vitro methods into the GHS scheme;
- A limitation to the practical application of AOPs in the regulatory context for concluding on (lack of) effect for any systemic toxicity endpoint is that metabolic (de)activation has to be considered. This means that biological models used to generate KE information need to be metabolically competent or complemented with reliable simulator of metabolism - which can be challenging.

* Guidance on IR&CSA - Chapter R.7a (europa.eu)

Example OECD-endorsed AOP 40: Skin Sensitisation

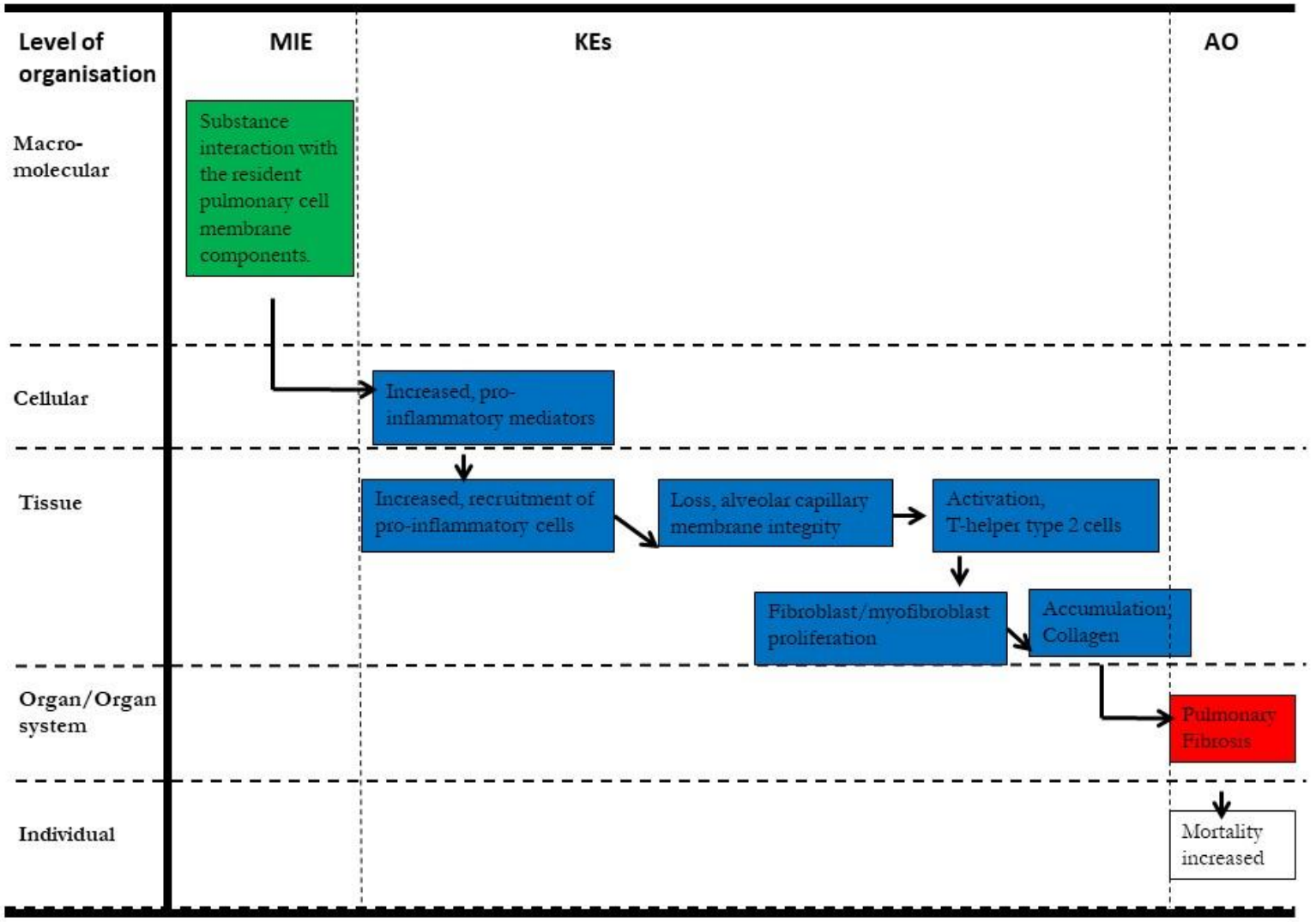


Source: https://aopwiki.org/aops/40

NAMs for Assessment of Skin Sensitisation

AOP KE covered	OECD TGs/ EU test method	Test method
MIE (KE1): covalent binding to skin proteins	OECD 442C (2020) / EC B.59 In chemico skin sensitisation	Direct Peptide Reactivity Assay (DPRA) Amino acid derivative reactivity assay (ADRA)
KE2: keratinocyte activation	OECD 442D (2018) / EC B.60 In vitro Skin Sensitisation Assays addressing the KE on keratinocyte activation	ARE-Nrf2 Luciferase KeratinoSens™ Test Method The ARE-Nrf2 luciferase LuSens test method
KE3: dendritic cell activation	OECD 442E (2018) / EC B.72 In vitro Skin Sensitisation Assays addressing the KE on activation of dendritic cells.	Human Cell Line Activation test (h-CLAT) U937 Cell line Activation Test (U-SENS™) Interleukin-8 Reporter Gene Assay (IL8-Luc assay)

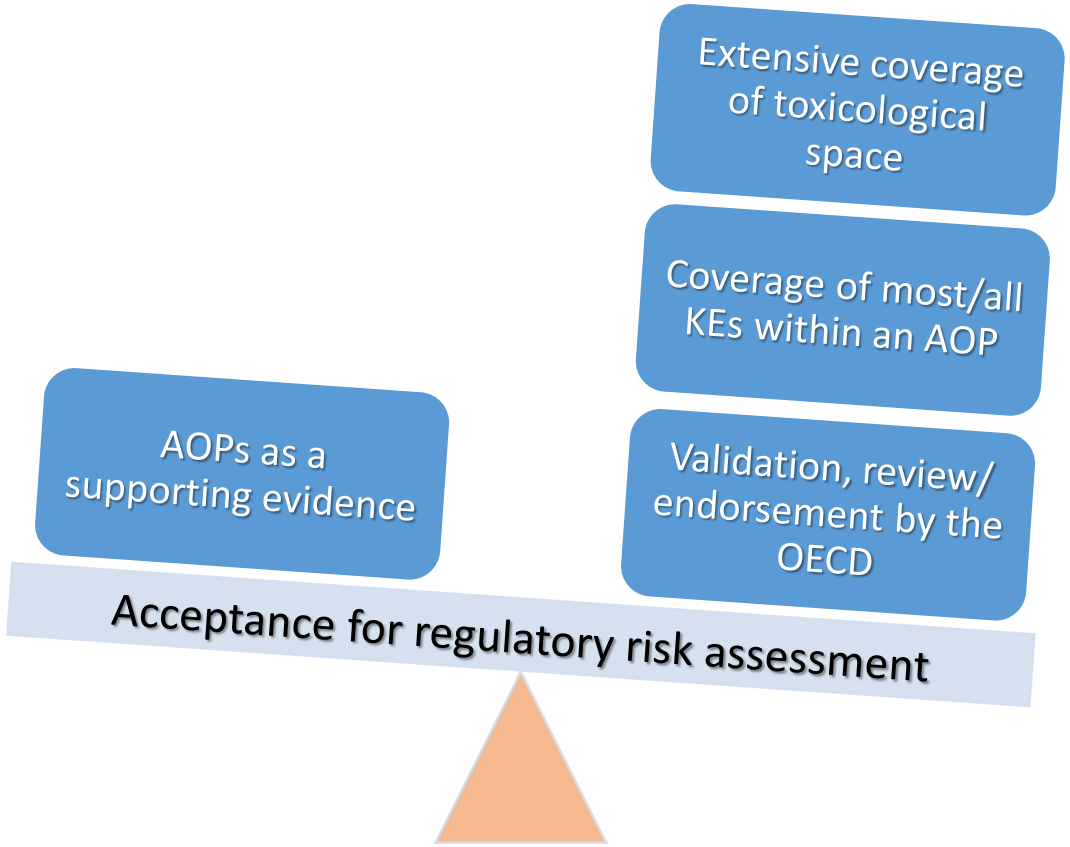
Example OECD-endorsed AOP 173: Pulmonary Fibrosis



AOPs: Work in Progress

- The need for the development and implementation of AOP across multiple endpoints features heavily in identified research needs of:
  - ECHA (industrial chemicals)*
  - EFSA (food/feed and agrochemicals)**
  - SCCS (cosmetics and personal-care products)***
- EFSA ongoing project into the development of AOPs for endocrine disrupting chemicals (EDCs)
- Other work into AOP development ongoing at various agencies

*https://echa.europa.eu/documents/10162/17228/key_areas_regulatory_challenge_en.pdf/fbaa76cf-acd0-0c8a-5dd7-3195379946aa
** Viviani et al. (2022): doi: 10.2903/sp.efsa.2023.EN-7748
*** SCCS Notes of Guidance (2023): https://health.ec.europa.eu/publications/sccs-notes-guidance-testing-cosmetic-ingredients-and-their-safety-evaluation-12th-revision_en


Extensive coverage of toxicological space
Coverage of most/all KEs within an AOP
AOPs as a supporting evidence
Validation, review/ endorsement by the OECD
Acceptance for regulatory risk assessment

Summary

- AOPs are a robust concept that allows linking molecular events with an adverse outcome;
- Regulatory risk assessors are more likely to accept a NAM if it addresses KE(s) of an AOP;
- Validated AOPs are more likely to be accepted - OECD endorsement gives a lot more credence;
- AOPs can be used to demonstrate presence of a toxicological hazard.
- To demonstrate the lack of effect, the AOP(s) need to cover sufficient toxicological space of the regulatory endpoint – otherwise, lack of an effect within known AOPs may not exclude toxicological effect via other (unknown) MoA;
- So far the use of AOPs to exclude a toxicological effect has been demonstrated only for a few less complex endpoints (e.g. skin sensitisation);
- The current limitations should not stop the development of a comprehensive AOP network with long-term aim to provide sufficient coverage of the toxicological space;
- More AOP developments are in the pipeline.

THANK YOU FOR YOUR ATTENTION