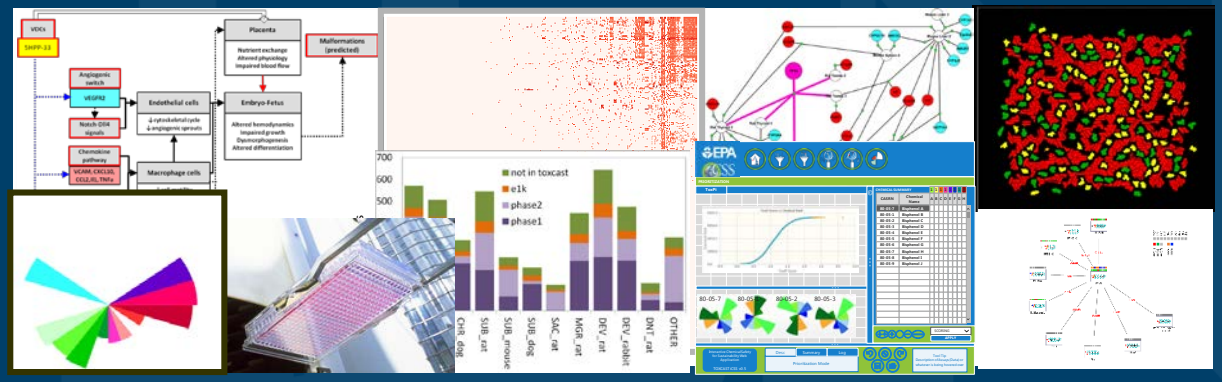
United States Environmental Protection Agency

Transitioning from AOP to IATA – Exploiting mechanistic insight for practical decision making



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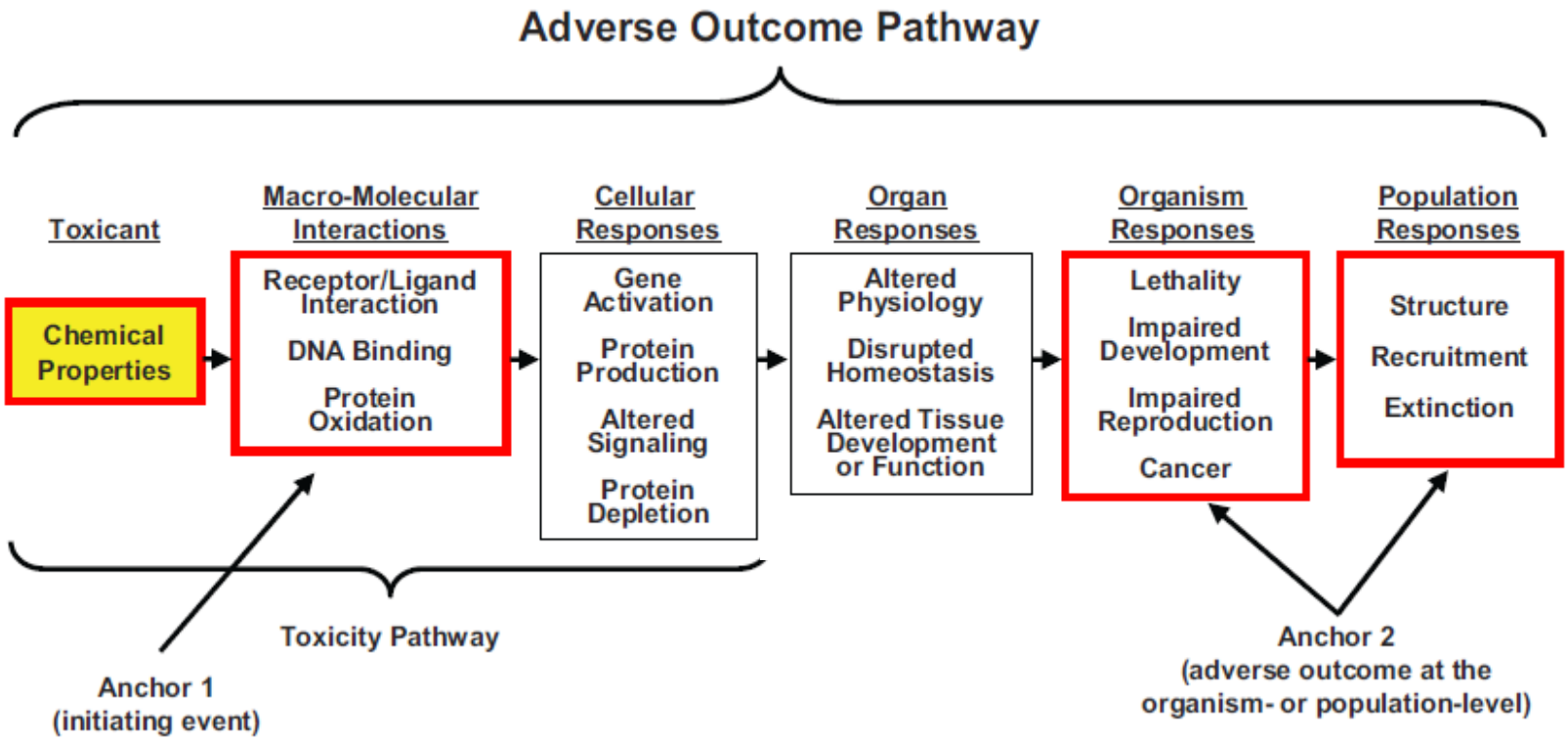
Outline

- AOPs
  - What information does an AOP provide?
  - Application of AOPs
- IATA
  - Definition
  - Conceptual workflow
  - IATA elements
- Scientific confidence considerations
- Case study example – skin sensitisation
- Take home messages

What information does an AOP provide?

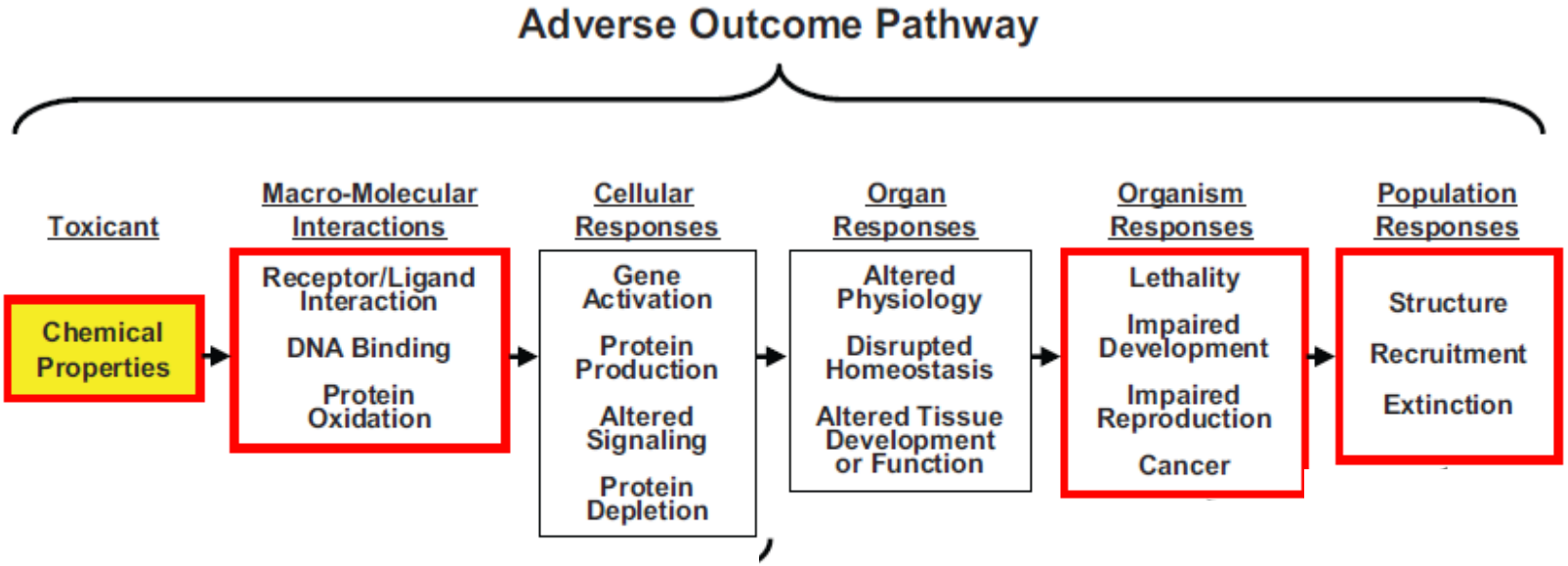
- OECD proposed a template for AOP generation
- The template standardises the documentation of an AOP including:
  - Background
  - Abstract
  - Summary of AOP and Key Event (KE) descriptions
  - Summary of Key Event Relationships (KERs) of the AOP
  - Assessment of the AOP
  - Potential Applications of the AOP

Using the AOP as a framework for answering toxicity questions



Ankley et al., Adverse outcome pathways: A conceptual framework to support ecotoxicology research and risk assessment. Environmental Toxicology and Chemistry, Vol. 29, No. 3, p. 730-741. 2010.

What resources exist to answer/deal with these questions?



QSAR Toolbox

In vitro/ Biochemical Assays

In vivo assays

'Omics technologies

- AOP Knowledge base

AOPs under OECD

- Three key avenues where AOPs will have significant impact:
  - Inform Test Guidelines use/development
  - Exploit the use of the QSAR Toolbox for grouping chemicals
  - Inform development of Integrated Approaches to Testing and Assessment

The Test Guideline Programme

- Identification of key events allows development of (screening) in vitro and ex vivo assays that detect direct chemical effects or responses at the cellular or higher levels of biological organisation
- By linking proposals for the development of in vitro test methods to key events in an AOP, the relationship to hazard endpoints relevant for regulatory purposes can be established

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Developing Chemical Categories

- Developing Chemical Categories (to facilitate read-across)
- The AOP can then be used to form categories by integrating knowledge of how chemicals interact with biological systems (i.e., the molecular initiating events) and in vitro and in vivo knowledge of the biological responses

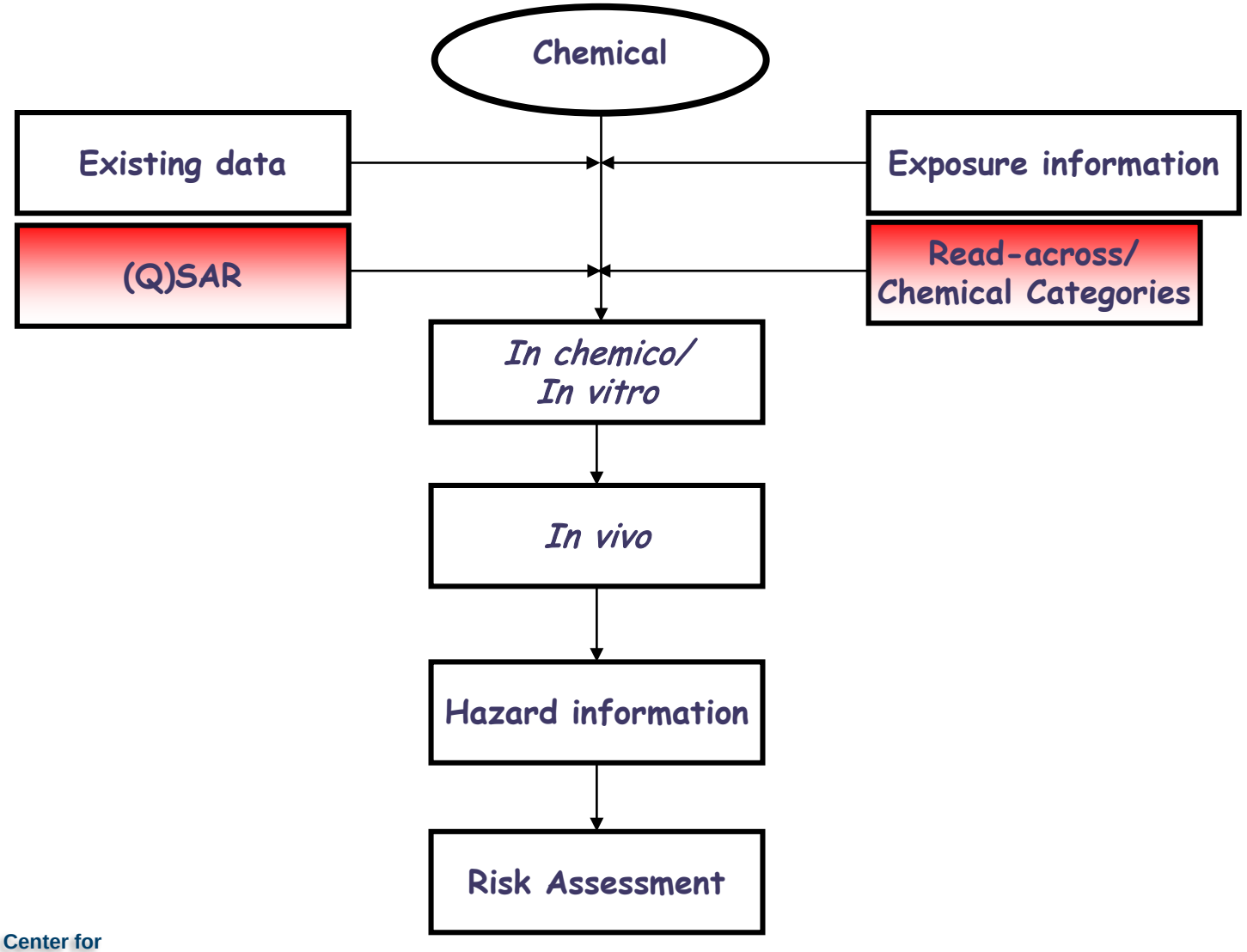
Development of Integrated Approaches

- An AOP can assist in determining what further information (and therefore, which test, if any), would increase the certainty of linking the initiating event and adverse effect(s). Moreover, a well established AOP can be used for species-to-species comparisons.

Integrated Approaches to Testing and Assessment (IATA)

- A means of integrating existing data and non-testing data together, determining what new information needs to be generated in order to make a decision with sufficient confidence for the purpose in mind

Conceptual IATA



IATA elements/components

- Typically characterised by their methodological approach or technology
- Historical information on the chemical of interest
  - Non-standard in vivo tests
- Information from "similar" chemicals (read-across)
- Predictions from other non-testing approaches such as (Q)SAR
- In chemico tests
- In vitro tests (e.g. HTS).
- Defined approaches comprising a data interpretation procedure (DIP)

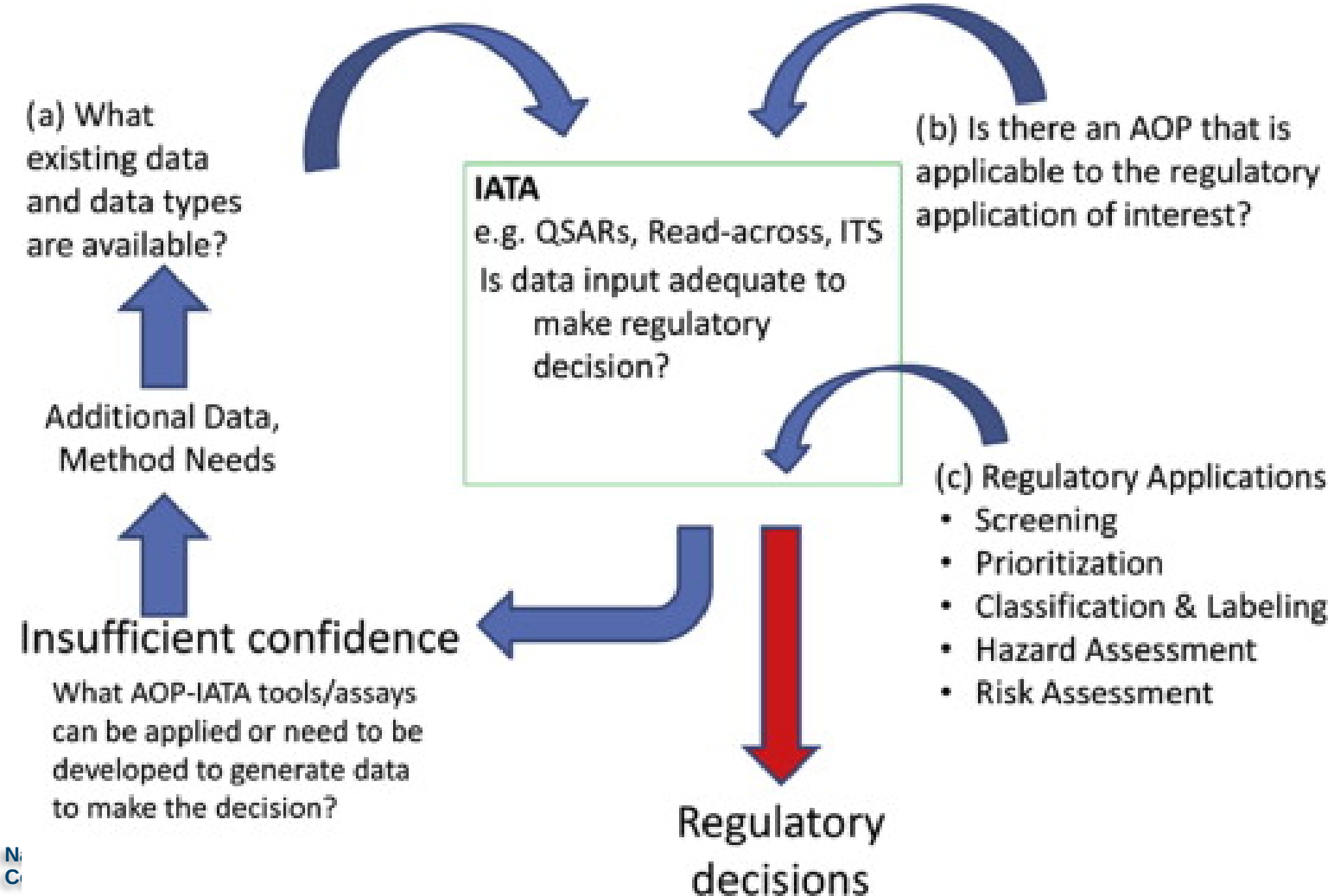
Defined Approaches to Testing and Assessment

- A defined approach to testing and assessment consists of a fixed data interpretation procedure (DIP) applied to data generated with a defined set of information sources to derive a result that can either be used on its own, or together with other information sources within an IATA, to satisfy a specific regulatory need.
- A defined approach to testing and assessment can be used to support the hazard identification, hazard characterisation and/or safety assessment of chemicals.

Evolution of IATA

- Greater emphasis on incorporating mechanistic information such as AOPs to inform the structure of the IATA
- i.e. the IATA elements describe the key events (KEs) they measure or compute, and the adverse outcomes (AOs) they can be used to (partially) predict.

Conceptual framework for an AOP-informed IATA to support regulatory decisions



Scientific confidence considerations for IATA

- **Validation principles:**
  - define the endpoint being assessed;
  - define the purpose/application for which the IATA is proposed;
  - describe the rationale underlying the construction of the IATA;
  - describe how the individual information sources constituting the IATA are integrated to derive the final prediction/assessment and,
  - describe the predictive capacity of the approach, the limitations in the application of the approach and the known uncertainties associated with the IATA application.
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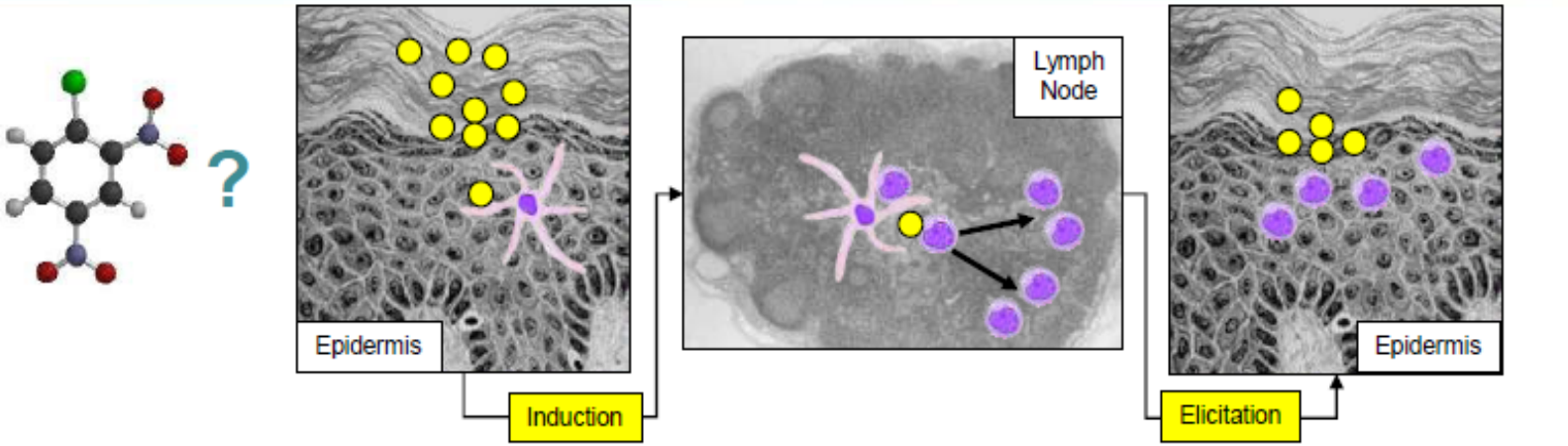
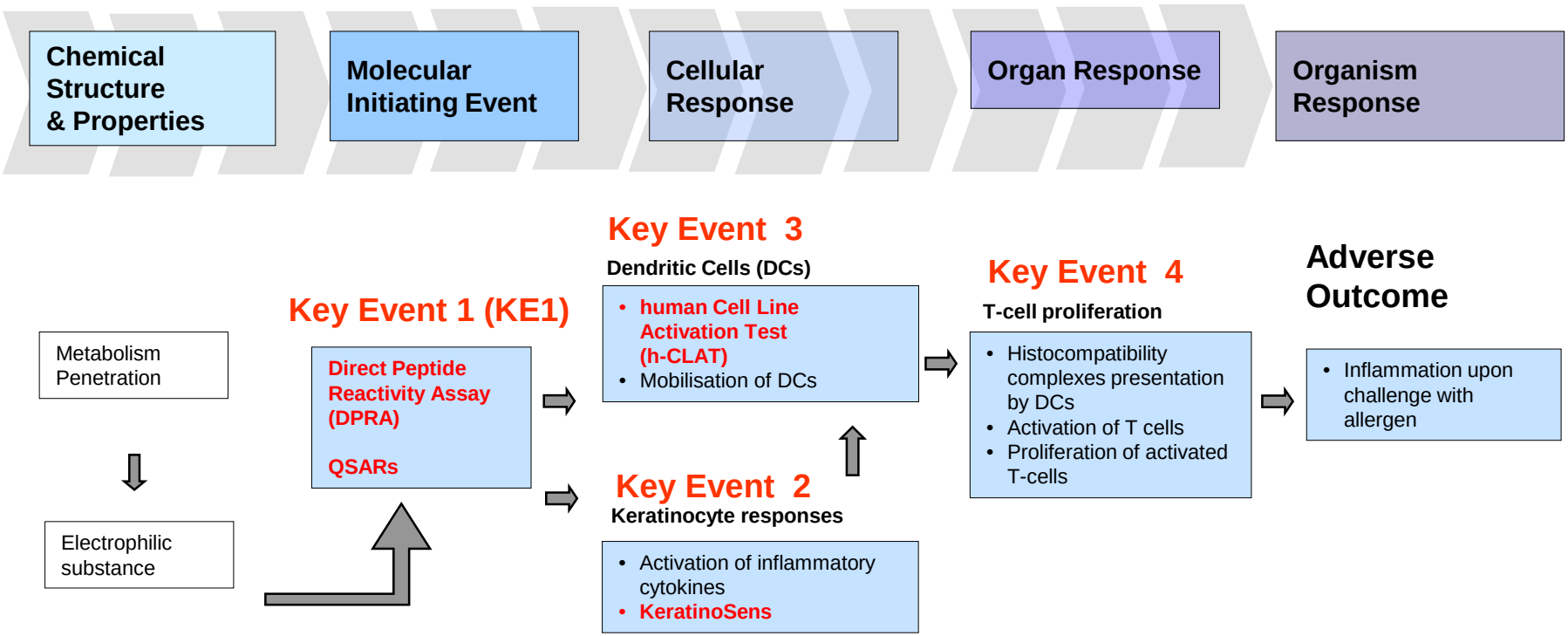
AOP for skin sensitisation (OECD, 2012)



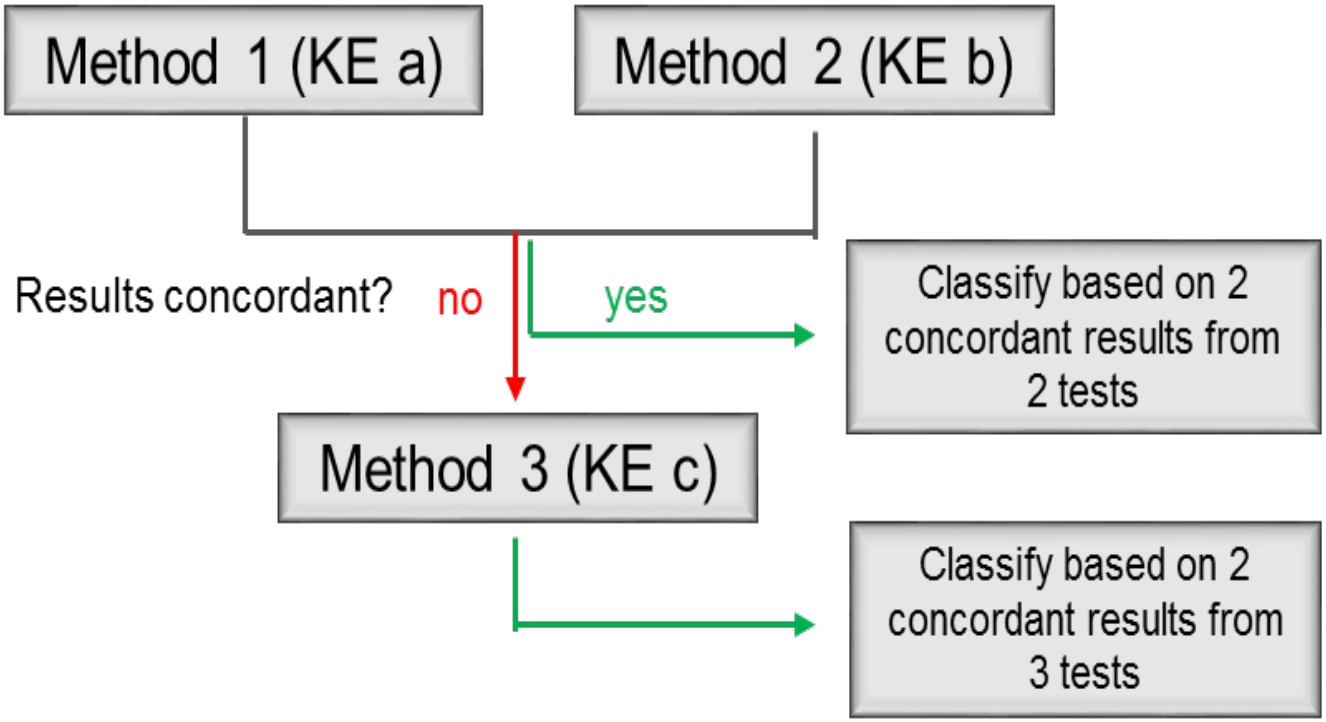
Chemical Structure & Properties	Molecular Initiating Event	Cellular Response	Organ Response	Organism Response
1. Skin Penetration 2. Electrophilic substance: directly or via auto-oxidation or metabolism	3-4. Haptenation: covalent modification of epidermal proteins	5-6. Activation of epidermal keratinocytes & Dendritic cells	7. Presentation of haptenated protein by Dendritic cell resulting in activation & proliferation of specific T cells	8-10. Allergic Contact Dermatitis: Epidermal inflammation following re-exposure to substance due to T cell-mediated cell death
Key Event 1	**Key Event 2 + 3**		**Key Event 4**	**Adverse Outcome**

AOP for skin sensitisation (SS) and assays mapped to KEs



AOP key event 1: Covalent interaction with cellular proteins	
	Non-testing methods • Protein binding/reactivity alerts (e.g. OECD Toolbox, Derek Nexus, Toxtree, TIMES-SS)[1] Testing methods • TG442C (Direct Peptide Reactivity Assay) • Adduct formation or relative reactivity rate, with or without metabolic activation, e.g: – Cor1C420 assay (Natsch and Gfeller, 2008) – PPRA (Gerberick et al., 2009) – Kinetic DPRA (Roberts and Natsch et al., 2009) – Glutathione depletion assay (Aptula et al., 2006; Schultz et al., 2005)
AOP key event 2: events in Keratinocytes	
Activation of biochemical pathways Pathways-associated gene expression Pathways-associated protein expression Release of pro-inflammatory mediators	Testing methods • TG 442D (ARE-Nrf2 Luciferase Test Method-KeratinoSens™) • LuSens (Ramirez et al., 2014, 2016) • AREc32 cell line assay (Natsch and Emter, 2008). • SENS-IS (Cottrez et al., 2015, 2016) • HaCaT gene signature (van der Veen et al., 2013) • SenCeeTox (McKim et al., 2012) • Epidermal Sensitization Assay (EpiSensA; Saito et al., 2013) • Proteomic signature in keratinocytes (Thierse et al., 2011)

Defined Approach (BASF) '2 out of 3 approach'



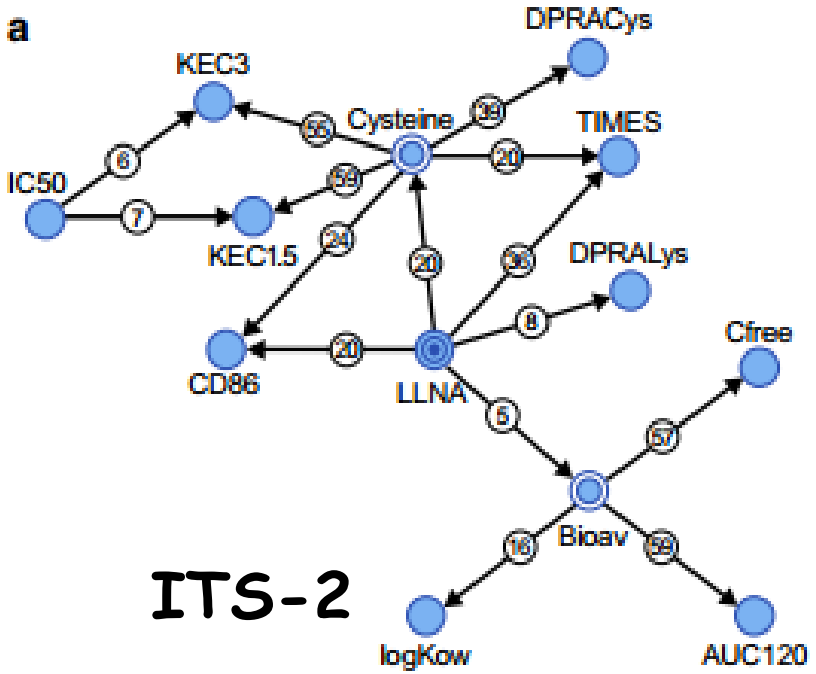
Developed to predict sensitisation potential to satisfy C&L needs

Defined Approach (BASF) '2 out of 3 approach'

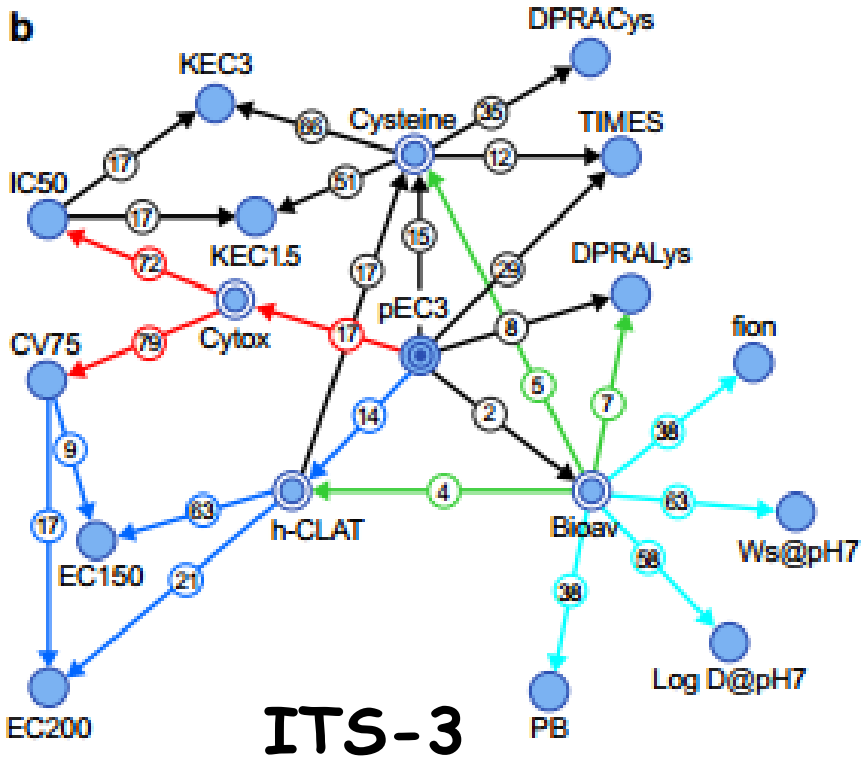
Compared to human data		n	Cooper statistics [%]				
			Sensitivity	Specificity	Positive predictive value	negative predictive value	Accuracy
animal test	LLNA	111	91	64	84	77	82
"2 out of 3 – Sens ITS"	DPRA + KeratinoSens + h-CLAT	101	90	90	96	79	90
	DPRA + KeratinoSens + (m)MUSST	95	84	100	100	70	88
	DPRA + LuSens + h-CLAT	90	90	89	95	80	90
	DPRA + LuSens + (m)MUSST	75	87	100	100	75	91

Compared to LLNA data		n	Cooper statistics [%]				
			Sensitivity	Specificity	Positive predictive value	negative predictive value	Accuracy
"2 out of 3 – Sens ITS"	DPRA + KeratinoSens + h-CLAT	180	82	72	89	59	79
	DPRA + KeratinoSens + (m)MUSST	171	79	77	90	59	78
	DPRA + LuSens + h-CLAT	133	83	78	91	64	82
	DPRA + LuSens + (m)MUSST	126	84	84	93	69	84

Bayesian Networks for Skin sensitisation



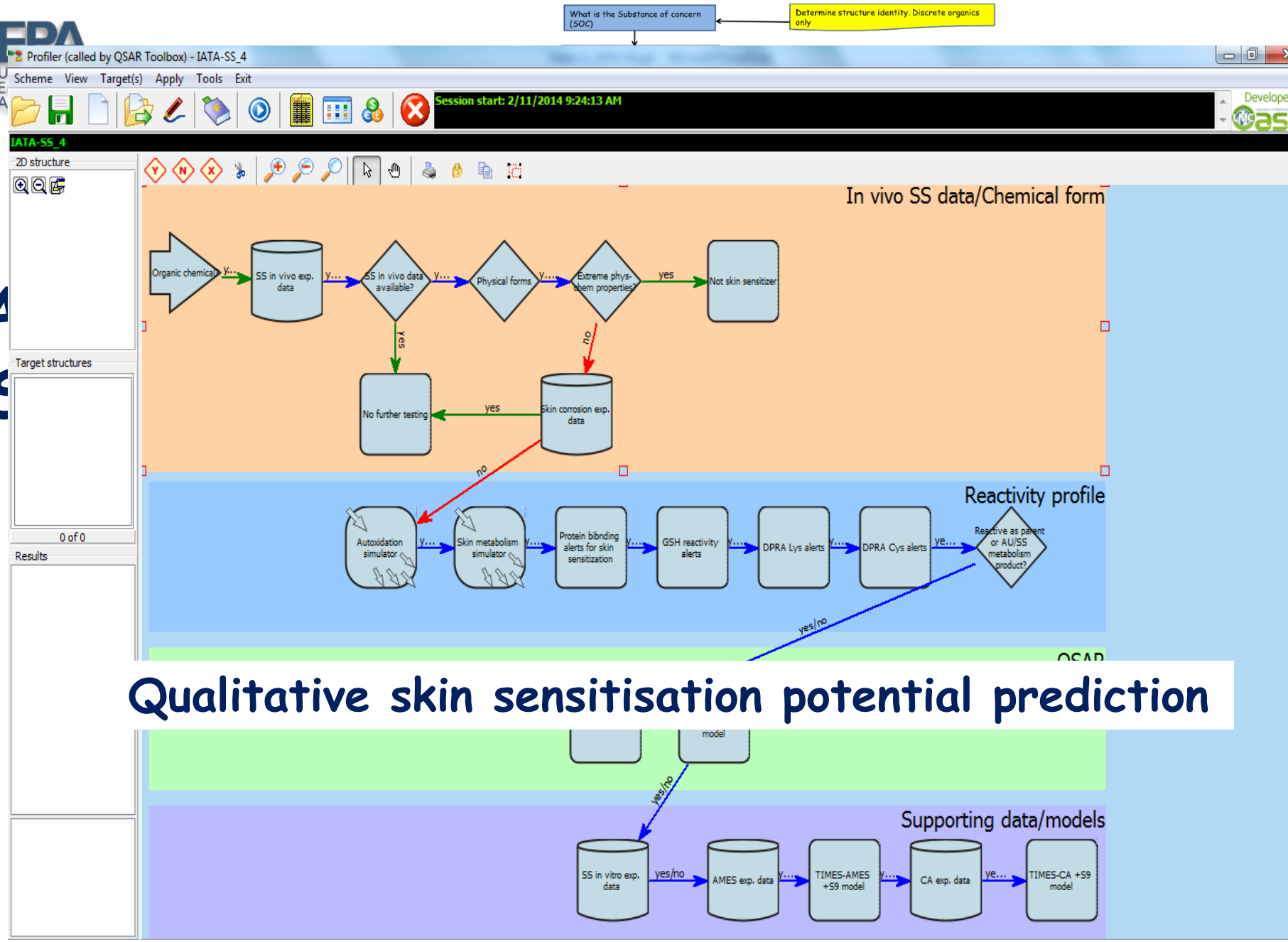
ITS-2



ITS-3

Developed to predict sensitisation potency

Jaworska et al, 2015



IA SS

Qualitative skin sensitisation potential prediction

Patlewicz et al, 2014

Component	Comments	Reference (scientific literature, Test Guidelines, Methods etc.)	Study result and/or positive (+ve)/negative (-ve) evidence obtained	Data reliability e.g. Klimisch	Data relevance including coverage/prediction of relevant parameters	Consistency with other information	Conclusive remarks (adequacy of information for given component)
Exposure information							
Degradation/Metabolism information e.g. degradation (including hydrolysis), metabolism, autoxidation	This could be simulated using tools such as the OECD QSAR Toolbox						
Non-testing approaches							

Partial WoE assessment table

Take home messages

- One of the potential applications of AOPs is to promote a step change in how IATA are constructed and used
- Scientific confidence needs to be considered for the AOP and associated IATA
- Decision context is critical
- KE information from different test methods or non-testing approaches are integrated together
  - One approach is by way of defined approaches which are underpinned by fixed data integration procedures
  - Alternatively qualitative approaches can be constructed where information is evaluated using a structured WoE table