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Regulatory acceptance & AOPs

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"In vitro effect-based bioanalysis technologies"

...could refer to

- ***in vitro*** **diagnostic medical devices/products (see next page)**

...or to

- **test systems for chemical contaminants in water, food, air... (I'll talk about these)**

Regulatory acceptance of biodetector data

- **Examples - dioxins screening in USA and EU**
- **Quantitative assessment; thresholds…**
- **The OECD AOP framework**

In vitro diagnostic medical devices/products...

- Hepatitis & HIV tests
- Clinical chemistry
- Coagulation test systems
- Urine test strips
- Pregnancy tests
- Blood sugar monitoring systems for diabetics

USA: IVDs are subject to the same pre- and postmarket controls as other medical devices.

http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/IVDRegulatoryAssistance/ucm123682.htm

EU: Directive 98/79/EC on *in vitro* diagnostic medical devices

http://ec.europa.eu/growth/single-market/european-standards/harmonised-standards/iv-diagnostic-medical-devices/index_en.htm

Bioanalytical test systems for chemical contaminants

Best example - dioxins in food and water

- **EU**
  Commission Regulation (EU) No 589/2014 of 2 June 2014 laying down methods of sampling and analysis for the control of levels of dioxins, dioxin-like PCBs and non-dioxin-like PCBs in certain foodstuffs

- **US EPA**
  Hazardous Waste Test Methods
  SW-846 Test Method 4435: Screening For Dioxin-Like Chemical (2008) Activity In Soils And Sediments Using The CALUX Bioassay And Toxic Equivalents (TEQs) Determinations

Example - food dioxins, EU

EU Regulation 589/2014 - assaying dioxins in food

- A [bioanalytical] screening method of analysis with widely acceptable validation and high throughput can be used to identify samples with significant levels of PCDD/Fs and dioxin-like PCBs.
- The levels in these screening samples need to be determined by a confirmatory method of analysis [e.g. GC-HRMS, GC-MS/MS].

Example - food dioxins, EU

EU Regulation 589/2014 - assaying dioxins in food

- Bioanalytical methods are not specific to those congeners included in the TEF-scheme. Other structurally related AhR-active compounds may be present in the sample extract which contribute to the overall response. Therefore, **bioanalytical results cannot be an estimate but rather an indication of the TEQ level in the sample**.

- Screening methods are suitable for demonstrating compliance or suspected non-compliance or exceedance of action levels and give an indication of the range of levels in case of follow-up by confirmatory methods.
  They are not suitable for purposes such as evaluation of background levels, estimation of intake, following of time trends in levels, or re-evaluation of action and maximum levels.

Example - soil dioxins, USA

Hazardous Waste Test Method SW-846 (2008): Screening for dioxin-like chemical activity in soils and sediments using the CALUX bioassay and Toxic Equivalents (TEQs) determinations

- By using the sample processing procedures in this method and an affinity column, polychlorinated biphenyls (PCBs) can be separated from chlorinated dioxins/dibenzofurans (PCDDs/PCDFs).
  This is the Dioxin/Furan- and PCB-specific (DIPS) analysis or the DIPS-CALUX® bioassay for dioxin-like chemicals.
- TEQ is defined by the response to the 2,3,7,8-TCC standard.
- Using this DIPS-CALUX bioassay it is possible to determine the portion of the total TEQ activity in a given sample that is due to each of these classes of compounds.
- For PCDDs/PCDFs, there is a high correlation between TEQs derived by this method and by GC/MS (R^2 = 0.9631).

https://www3.epa.gov/wastes/hazard/testmethods/sw846/pdfs/Method%204435,%20Revision%200%20-%202008.pdf

Thresholds - "Effect-based trigger values"

Escher et al (2015)

- Modern reporter gene assays and other cellular bioassays are so sensitive that even very clean waters often induce detectable responses *in vitro*.
- High sensitivity is an advantage from the point of view of achieving the highest possible sensitivity and in the assessment of treatment efficacy (i.e., water treatment plants).
- In terms of water quality, however, **a cellular response does not translate directly into a toxicological effect**. A ‘simple’ yes or no answer will be defined by the sensitivity of the test and is not sufficient to evaluate if a water is “good” or “bad”. Therefore, trigger values are required that differentiate an acceptable response from an unacceptable response.

Effect-based trigger values for in vitro bioassays: Reading across from existing water quality guideline values. Escher BI, Neale PA, Leusch FD. Water Res. 2015 Sep 15;81:137-48.

Effect-based trigger values for *in vitro* bioassays

Escher et al (2015)

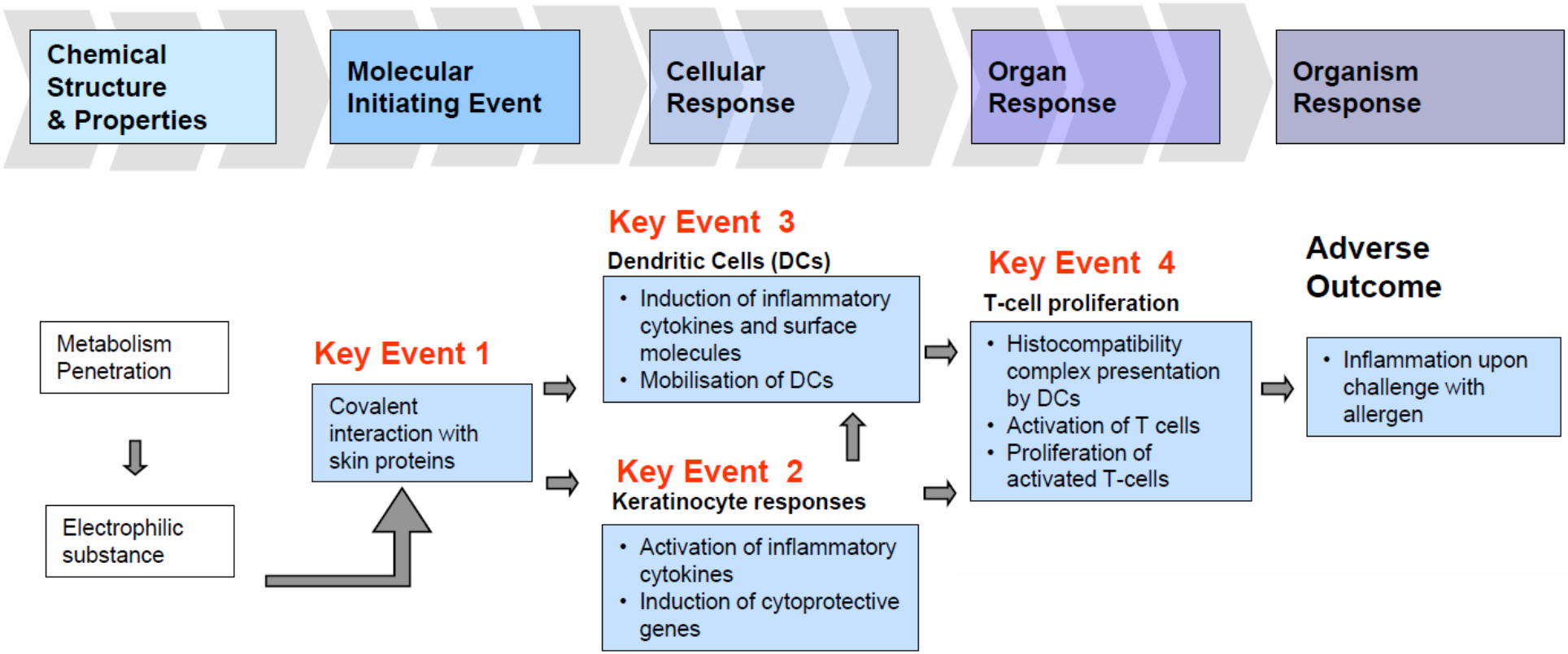
- 10 reference chemicals (metolachlor, malathion, TCDD, carbaryl, diuron, parathion, testosterone, 17b-estradiol, progesterone, dexamethasone), and 9 water samples
- 18 in vitro bioassays (PXR, PPARg, AhR, AR, ER, PR, GR, algal photosynthesis and AChE inhibition).
- 11 specific effect-based trigger bioanalytical equivalent concentrations (EBT-BEQ) were derived from the 18 bioassays.
- The EBT-BEQ values were comparable to those derived by other endpoint-specific approaches, although **the match with existing guideline values was in many cases rather poor**.
- We hope to triggers a discussion on **what bioanalytical tools mean** and **what would be the thresholds between acceptable and unacceptable water quality**.

Effect-based trigger values for in vitro bioassays: Reading across from existing water quality guideline values. Escher BI, Neale PA, Leusch FD. Water Res. 2015 Sep 15;81:137-48.

How to make *in vitro* bioassays acceptable;

the OECD Adverse Outcome Pathways framework

Skin Sensitisation: First Case of AOP-driven IATA Development



The Adverse Outcome Pathway for Skin Sensitisation Initiated by Covalent Binding to Proteins; Part 1: Scientific Evidence. OECD Series on Testing and Assessment No.168 ENV/JM/MONO(2012)10/PART1

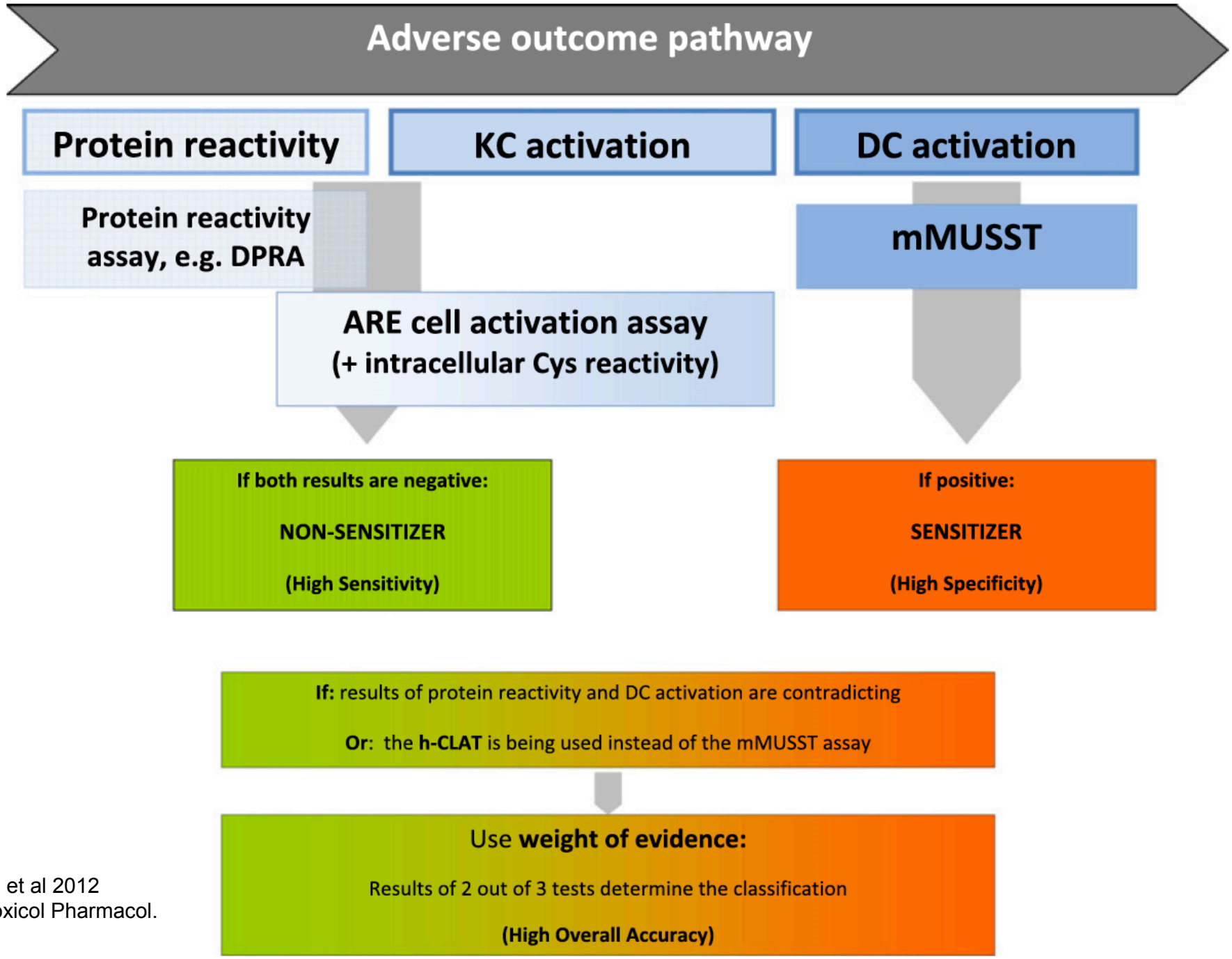
João Barroso, EU Joint Research Centre JRC. Integrated Approaches to Testing and Assessment (IATA) – ex. Skin Sensitisation. 10th EPAA Annual Conference, Brussels, 2014. https://circabc.europa.eu/sd/a/e19c814a-39ac-43fa-8f75-5715185f16d9/03-barroso.pdf

OECD *in vitro* skin sensitization testing

Regulatory *in vitro* identification of human skin sensitizers uses an AOP-based combination of assays:

- **covalent protein binding**
  (In Chemico Direct Peptide Reactivity Assay; OECD 442C)
- **keratinocyte inflammation**
  (ARE-Nrf2 Luciferase; OECD 442D; KeratinoSens™)
- **dendritic cell activation**
  (human Cell Line Activation Test h-CLAT or modified myeloid U937 dendritic cell activation-based skin sensitization test mMUSST)

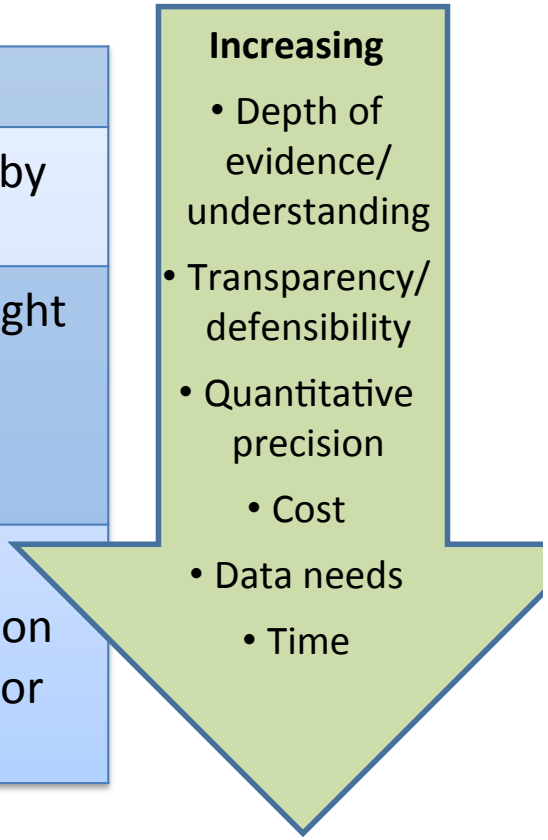
The Adverse Outcome Pathway for Skin Sensitisation Initiated by Covalent Binding to Proteins; Part 2: Use of the AOP to Develop Chemical Categories and Integrated Assessment and Testing Approaches. OECD Series on Testing and Assessment No.168 ENV/JM/MONO(2012)10/PART2



Bauch C et al 2012
Regul Toxicol Pharmacol.

AOP - How much detail?

AOP level	Characteristics
Putative	Hypothesized set of KEs and KERs primarily supported by biological plausibility and/or statistical inference
Formal	Include assembly and evaluation of the supporting weight of evidence – developed in AOP knowledgebase in accordance with internationally-harmonized OECD guidance
Quantitative	Supported by quantitative relationships and/or computational models that allow quantitative translation of key event measurements into predicted probability or severity of adverse outcome



- All levels are potentially useful
- The level of development desired or required depends on the application

Conclusion

- **With reference to human health risks, an *in vitro* bioassay is only useful if it measures something (a "key event readout") which is plausibly related to a defined adverse outcome.**
- **Reliable measurement of early key events in an established AOP will facilitate regulatory acceptance of the test system as relevant to hazard identification and, if triggering thresholds are characterized, to quantitative risk assessment.**

"It takes a lot of hard work to make something simple, to truly understand the underlying challenges and come up with elegant solutions."

Steve Jobs (biography by W. Isaacson, Simon & Schuster 2011)