The European Commission's science and knowledge service

Joint Research Centre

Últimos Avances en Validaciones

Pilar Prieto

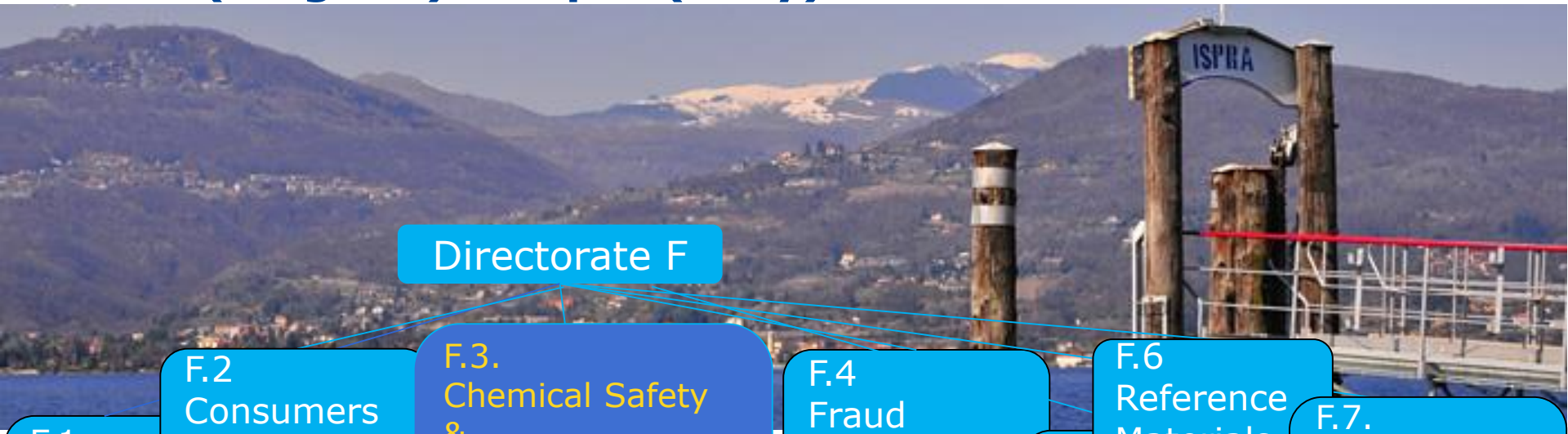
REMA, Actividad de los Comités Europeos relacionados con Riesgos Químicos
12 abril 2018

European Commission

Joint Research Centre (JRC), Directorate F:

Health, Consumers and Reference Materials

Geel (Belgium) & Ispra (Italy)



Directorate F

- F.1 Health in Society
- F.2 Consumers Products Safety
- F.3. Chemical Safety & Alternative Methods, Ispra
  Head of Unit: Maurice Whelan
- F.4 Fraud Detection & Prevention
- F.5 Food & Feed Compliance
- F.6 Reference Materials
- F.7. Knowledge for Health & Consumer Safety

European Union Reference Laboratory for Alternatives to Animal Testing

Established under the Directive 2010/63/EU on the protection of animals used for scientific purposes

Duties and tasks*

- Guide research on alternative methods
- Coordinate validation within the EU
- Disseminate information on the 3Rs
- Promote stakeholder dialogue
- Promote international acceptance

** Article 48 of the Directive, Annex VII*

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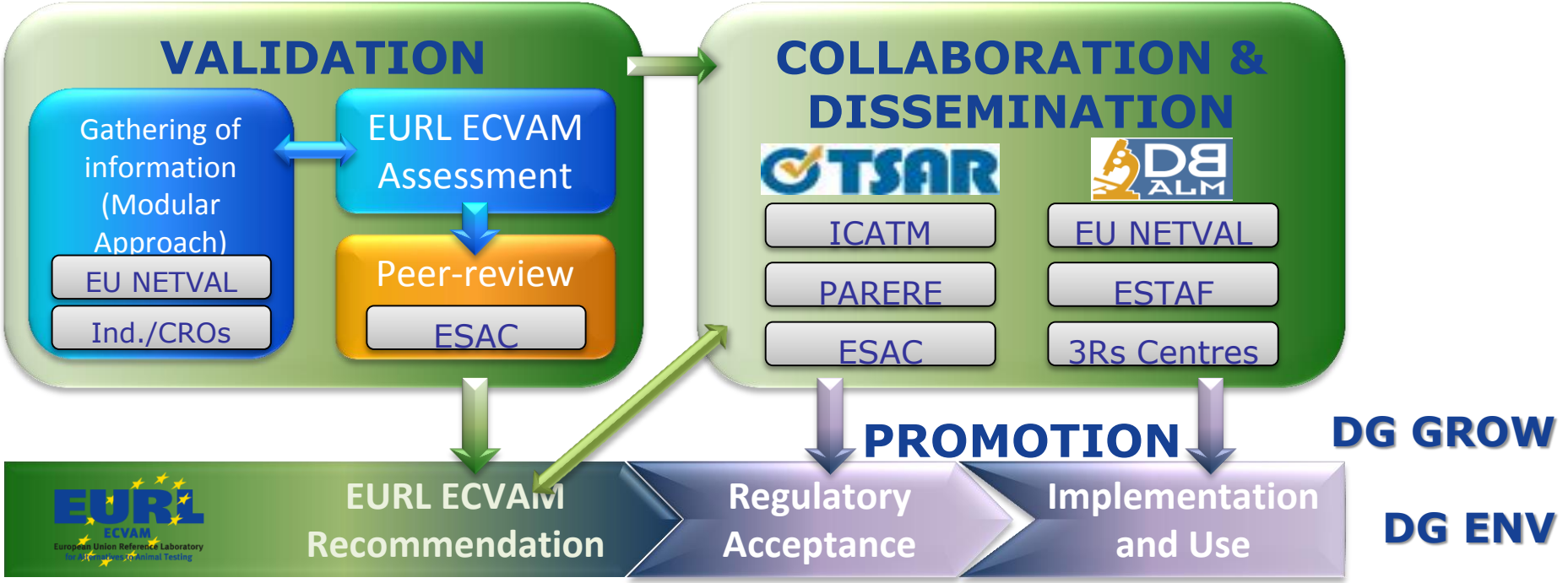
Objectives

Predict
adverse outcome
or disease

Validate
new *in vitro* methods

Disseminate
knowledge of alternative
approaches

The role of EURL ECVAM in the evolution of regulatory methods



Why do we need validation?

- ✓ Essential prerequisite for regulatory acceptance of a method/approach
- ✓ Regulators and end-users need to be confident and convinced that an alternative approach can provide a similar level of protection of human health or the environment when compared to traditional methods

Crisis in reproducibility

Reliability

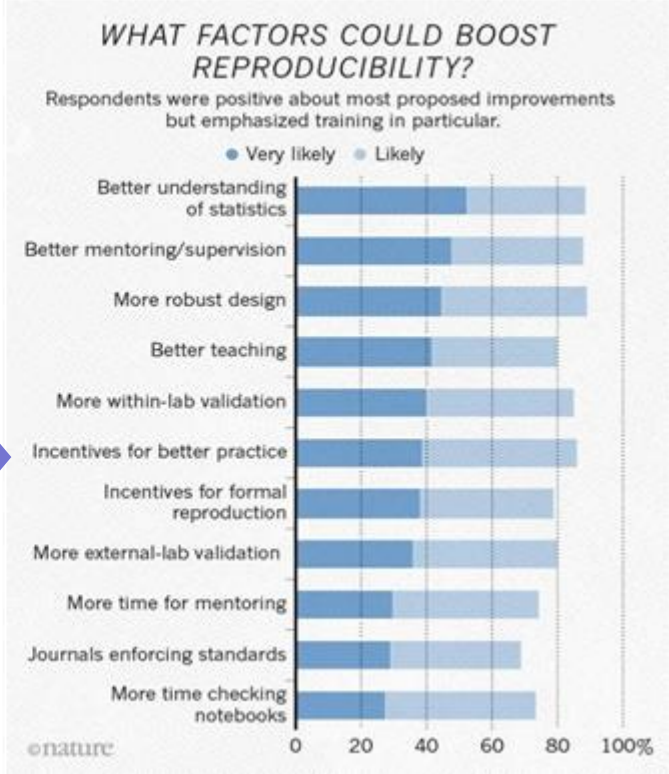
nature International weekly journal of science

Is there a reproducibility crisis in science?

More than 70% of researchers have tried and failed to reproduce another scientist's experiments

More than half have failed to reproduce their own experiments

Nature 533, 452–454 (2016)



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Validation Process

ATLA 18, 313–337, 1990 313

Report and Recommendations of the CAAT[1]/ERGATT[2] Workshop on the Validation of Toxicity Test Procedures[3]

Michael Balls[4], Bas Blaauboer[5], David Brusick[6], John Frazier[7], Denise Lamb[8], Mark Pemberton[9], Christoph Reinhardt[10], Marcel Roberfroid[11], Herbert Rosenkranz[12], Beat Schmid[13], Horst Spielmann[14], Anna-Laura Stammati[15] and Erik Walum[16]

Unclassified ENV/JM/MONO(2005)14

Organisation de Coopération et de Développement Economiques
Organisation for Economic Co-operation and Development 18-Aug-2005

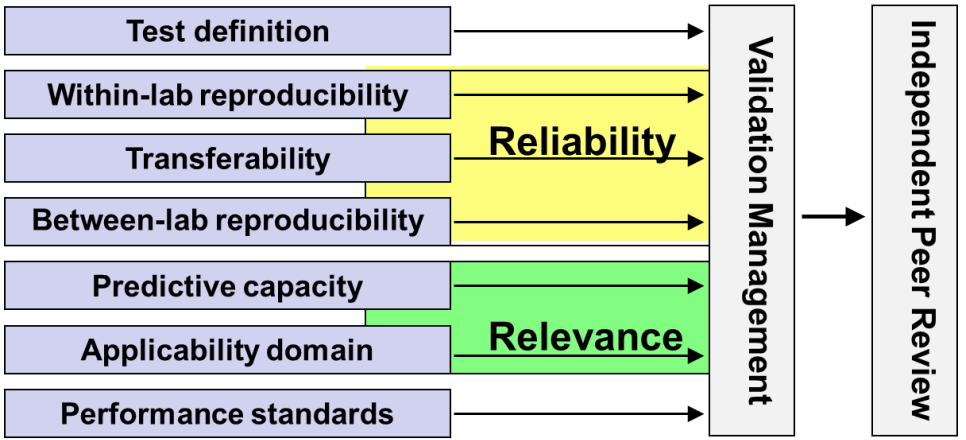
English - Or. English

ENVIRONMENT DIRECTORATE
JOINT MEETING OF THE CHEMICALS COMMITTEE AND
THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY

OECD SERIES ON TESTING AND ASSESSMENT
Number 34

GUIDANCE DOCUMENT ON THE VALIDATION AND INTERNATIONAL ACCEPTANCE OF NEW OR UPDATED TEST METHODS FOR HAZARD ASSESSMENT

Modular Approach to Validation



Hartung et al. (2004). ATLA 32, 467-472

"No sequential assessment needed"

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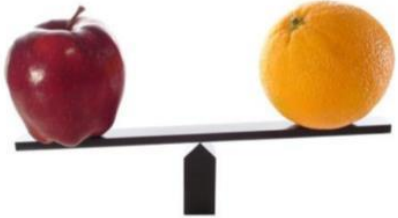
Current challenges in validation

Benchmarks

relevant ref. data

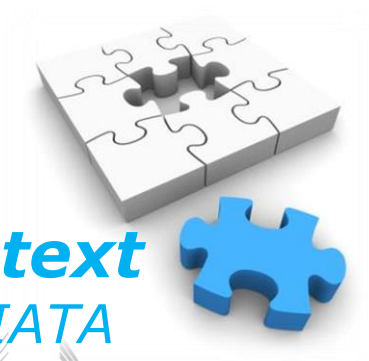


Information requirements

No one-to-one replacement methods!

human relevance

Time Constraints



Context

e.g. IATA

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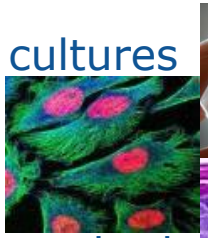
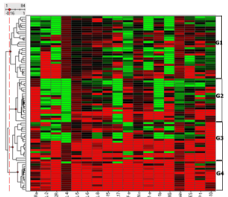
Predictive toxicology

Organ-on-a-chip

Cell cultures



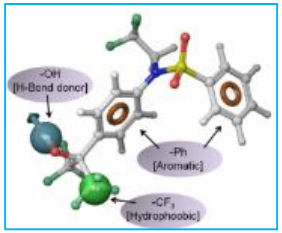
'OMICS



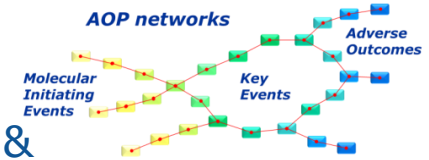
High content imaging

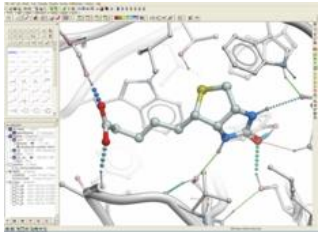
HTS

3D tissues



Cheminformatics & QSAR modelling





DATA INTEGRATION

PREDICT SAFETY

Exposure modelling

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Integration: an evolving concept…

Definition/explanation of ITS or IATA	Reference
"An integrated testing strategy is any approach to the evaluation of toxicity which serves to reduce, refine or replace an existing animal procedure, and which is based on the use of two or more of the following: physicochemical data, *in vitro* data, human data (for example, epidemiological, clinical case reports), animal data (where unavoidable), computational methods (such as quantitative structure-activity relationships [QSAR]) and biokinetic models."	Blaauboer et al. (1999)
"In the context of safety assessment, an Integrated Testing Strategy is a methodology which integrates information for toxicological evaluation from more than one source, thus facilitating decision-making. This should be achieved whilst taking into consideration the principles of the Three Rs (reduction, refinement and replacement)."	Kinsner-Ovaskainen et al. (2009)
"ITS can be described as combinations of test batteries covering relevant mechanistic steps and organised in a logical, hypothesis-driven decision scheme, which is required to make efficient use of generated data and to gain a comprehensive information basis for making decisions regarding hazard or risk. We approach ITS from a system analysis perspective and understand them as decision support tools that synthesise information in a cumulative manner and that guide testing in such a way that information gain in a testing sequence is maximised.	Jaworska and Hoffmann (2010)
"In the context of safety assessment, an ITS is a methodology integrating information from several sources of toxicological evaluation allowing appropriate decision making."	De Wever et al. (2012)
"An integrated test strategy is an algorithm to combine (different) test result(s) and, possibly, non-test information (existing data, *in silico* extrapolations from existing data or modeling) to give a combined test result. They often will have interim decision points at which further building blocks may be considered."	Hartung et al. (2013)

- Testing strategies
- Intelligent testing strategies
- Testing and assessment strategies
- Toxicity testing strategies
- More…

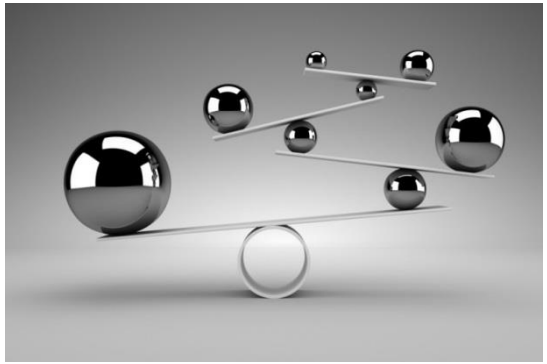
C. Eskes, M. Whelan (eds.), *Validation of Alternative Methods for Toxicity Testing*, Advances in Experimental Medicine and Biology 856,

…now embraced at global regulatory level

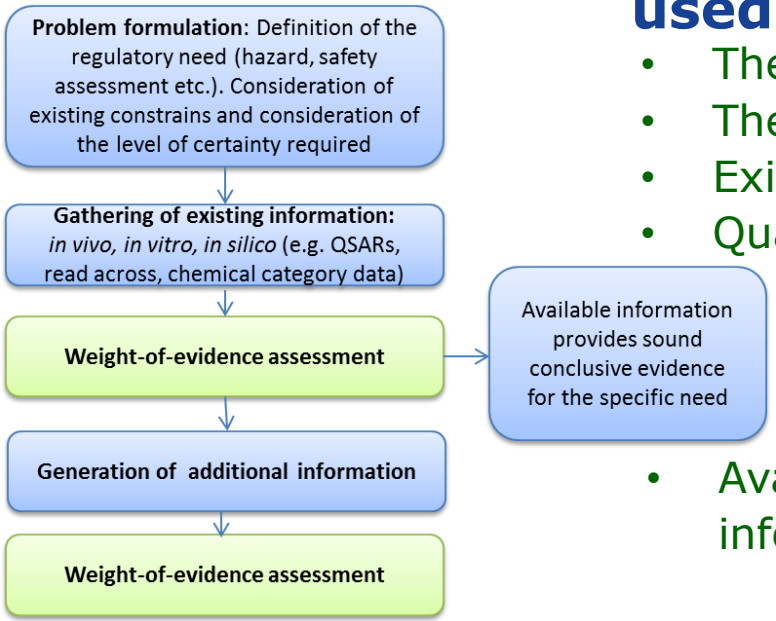
Integrated Approaches to Testing and Assessment (IATA)

OECD Guidance Document No. 255

A framework for hazard identification, hazard characterisation and/or safety assessment of chemicals based on multiple information sources, i.e. physicochemical properties, non-testing methods (QSARs, read-across), testing methods (*in chemico*, *in vitro*, *in vivo*)



IATA generic framework



Extent and type of information sources used within an IATA depend on:

- The chemical under investigation
- The specific regulatory need
- Existing constrains
- Quality and adequateness of existing information
- Availability of methods to generate additional information

There are potentially many different ways of applying an IATA for a given chemical and regulatory need

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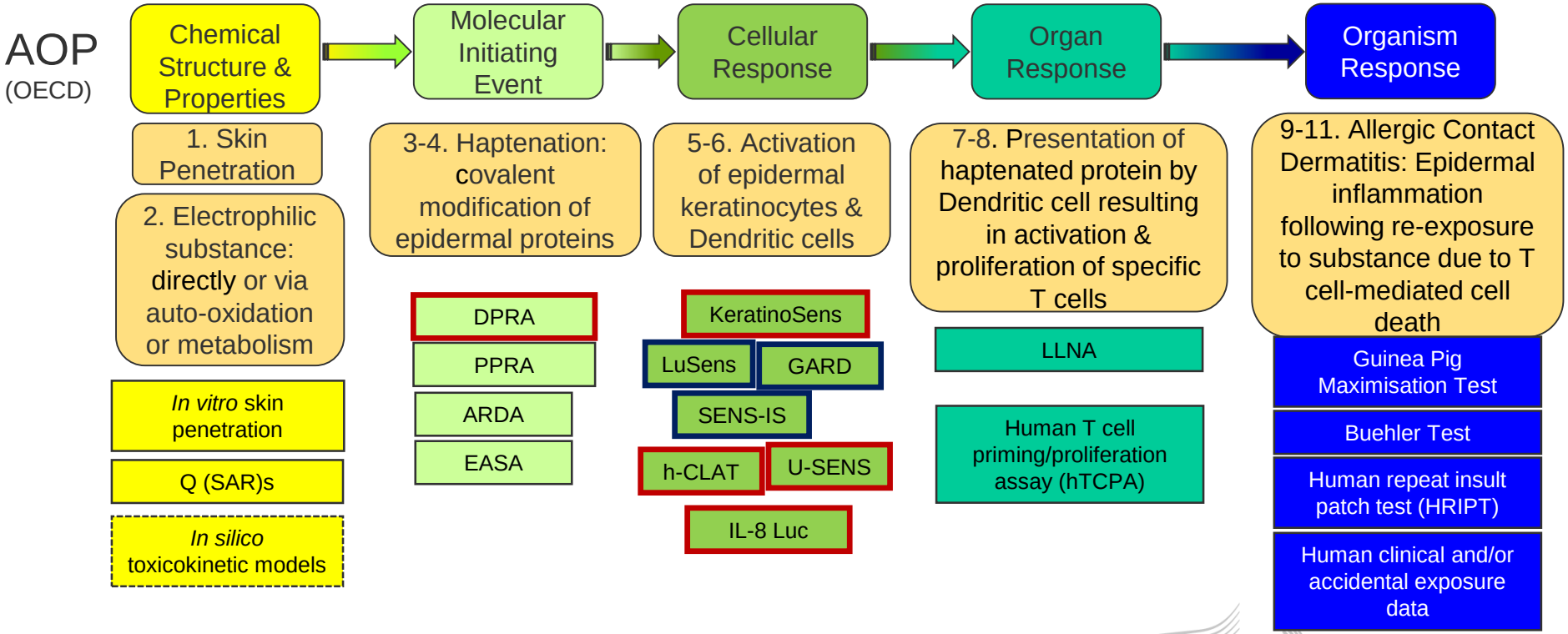
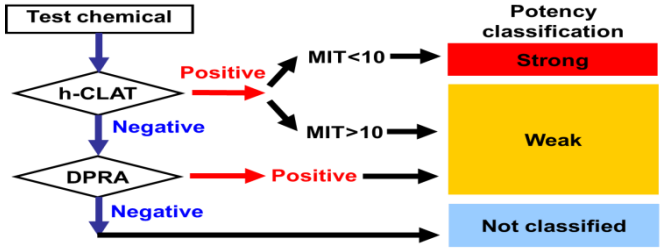
Skin sensitisation: information sources by key event



Many possibilities of combining information



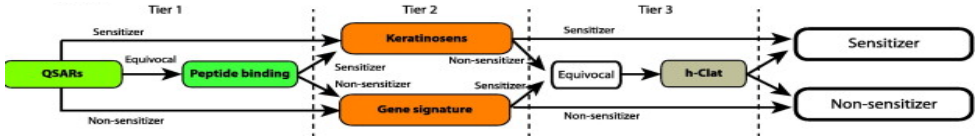
Takenouchi et al. (2015) J. Appl. Toxicol.: STS & ITS

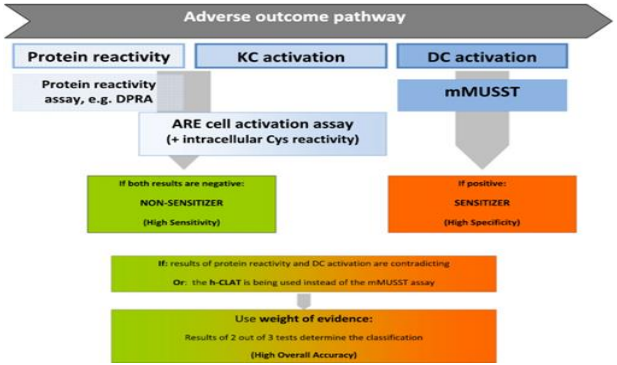
Score	h-CLAT MIT	DPRA depletion	DEREK
3	≤10 µg/mL	≥42.47%	-
2	>10, ≤150 µg/mL	≥22.62, <42.47%	-
1	>150, ≤5000 µg/mL	≥6.376, <22.62%	Alert
0	not calculated	<6.376%	No alert

Potency: Total battery score

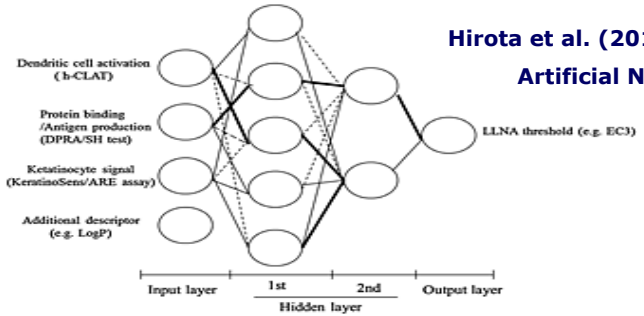
Strong :	7
Weak :	2-6
Not classified :	0-1

Van der Veen et al. (2014) Regul. Toxicol. Pharmacol.: STS

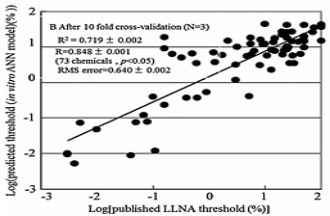


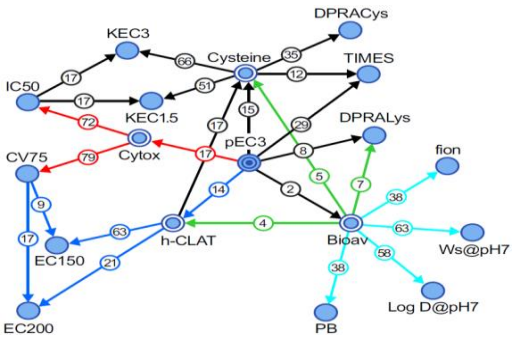


Bauch et al. (2012) Regul. Toxicol. and Pharmacol.: 2 out of 3



Hirota et al. (2015) J. Appl. Toxicol.: Artificial Neural Network



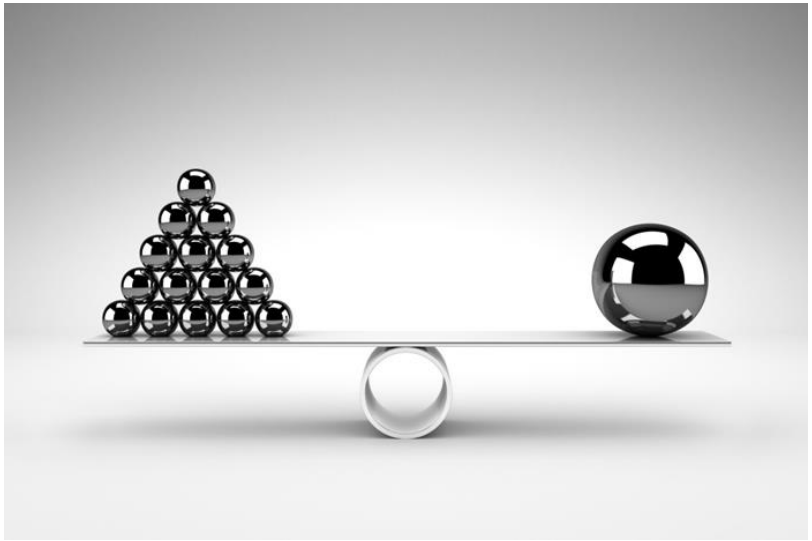


Jaworska et al. (2015) Arch. Toxicol.: Bayesian Network

Defined Approaches

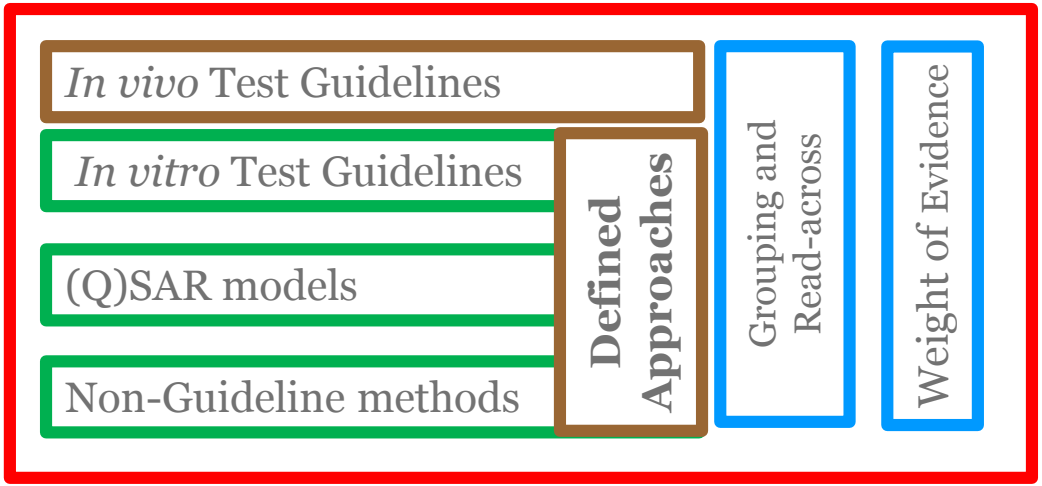
- A Defined Approach consists of a fixed data interpretation procedure (DIP) applied to data generated with a defined set of information sources (formalised decision-making approach)

- The result can either be used on its own, or together with other information sources within an IATA



OECD Guidance Document No. 255

IATA & Defined Approaches (DA)



Casati et al. (2018). Arch. Toxicol. 92 (2), 611–617.

OECD Guidance Document No. 256 (DAs for skin sensitization)

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Validation of alternative methods in a regulatory context …the 3Ps

*While the **purpose** and **principles** of validation remain relatively constant, the **process** of validation needs to evolve to keep pace with scientific progress and to benefit from it.*

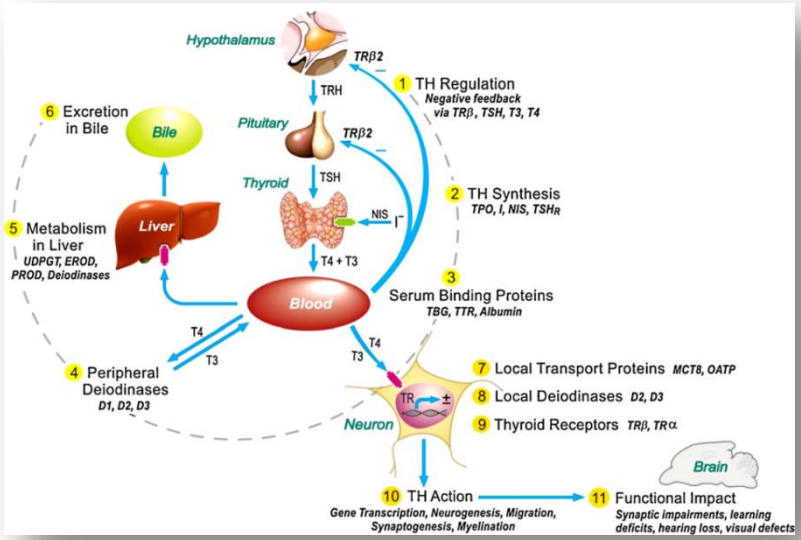
One size doesn't fit all.

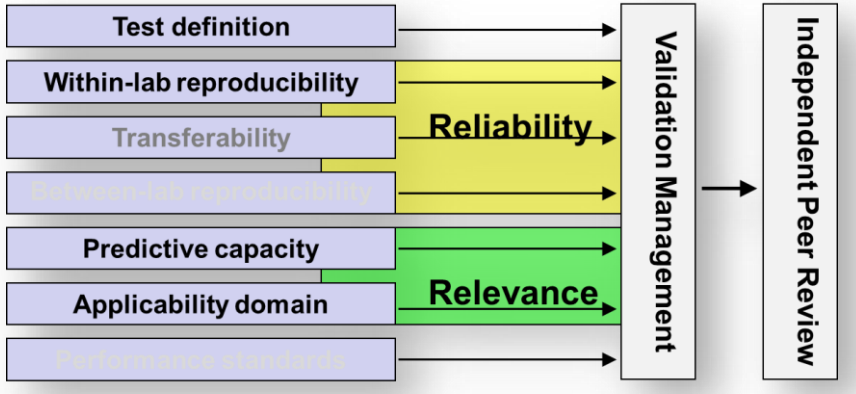
Approach needs to be fit for purpose!

Evaluation, validation and translation into regulation of *in vitro* test methods

Thyroid hormone signalling disruption

Exploring more efficient ways to validate mechanistic methods





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Use of benchmark animal data

- **Variability of animal data** should be characterised and considered when evaluating alternative approaches

- **Relevance to predict human effects** should also be considered, where possible (in the case of human health endpoints)

How should we interpret performance statistics of non-animal approaches? What does it mean that that a certain in vitro method or approach has e.g. 80% accuracy?

Use of benchmark animal data

Example: Local Lymph Node Assay (LLNA)

Toxicology in Vitro 34 (2016) 220–228

Contents lists available at ScienceDirect

Toxicology in Vitro

journal homepage: www.elsevier.com/locate/toxinvit

Analysis of the Local Lymph Node Assay (LLNA) variability for assessing the prediction of skin sensitisation potential and potency of chemicals with non-animal approaches

Coralie Dumont, João Barroso, Izabela Matys, Andrew Worth, Silvia Casati *

Joint Research Centre, European Commission, Ispra, Italy

"A level of accuracy of non-animal approaches for identifying non-sensitisers, moderate sensitisers and strong sensitisers of 70%, 70% and 80%, respectively, would be comparable to the performance of the LLNA"

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Validation of alternative methods: the way forward

- Method validation continues to be necessary in order to increase trust and facilitate regulatory acceptance (e.g., OECD)
- Validation should continue being fit for purpose, e.g. accommodate a shift in emphasis from individual methods to integration of multiple information sources
- Used as a tool to characterise the performance of a method or DA and the uncertainty associated with their predictions
- Important to characterise (human) relevance and uncertainty of reference *in vivo* method
- Compare uncertainty of alternative approaches with uncertainty of standard *in vivo* methods

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