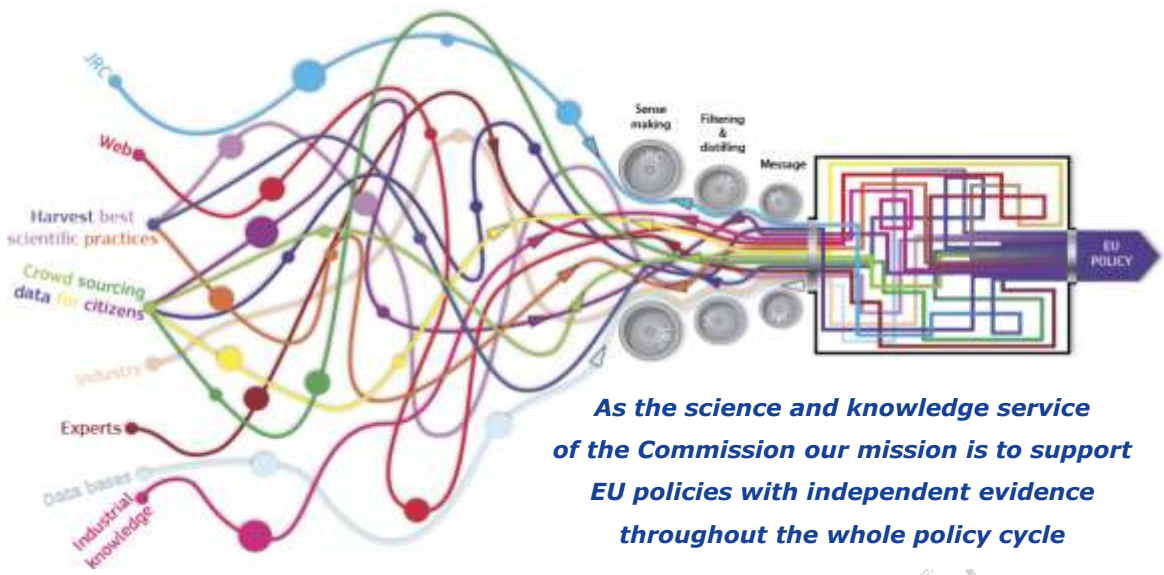
**The European Commission's science and knowledge service**

Joint Research Centre

# Incorporating novel methods into integrated approaches to testing and assessment of chemicals

Maurice WHELAN

February 2018




***As the science and knowledge service of the Commission our mission is to support EU policies with independent evidence throughout the whole policy cycle***

# CHEMICALS

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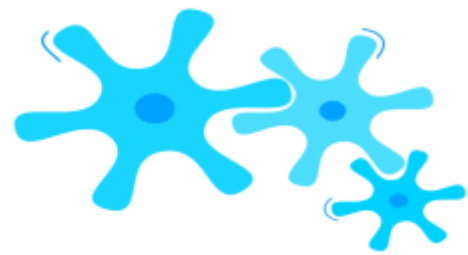
## General aims of chemicals legislation

- protection of human health and the environment
- efficient functioning of markets and trade
- innovation, competitiveness and sustainability

***RISK***
*Assessment*
*Management*
*Communication*



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# EU chemicals acquis

*… over 100 pieces of legislation !*

Regulation 1907/2006 on registration, evaluation, authorisation and restriction of chemical substances **(REACH)**

Community Strategy on combined exposures **'Mixtures'**

*Risk management based on* ***generic risk considerations*** *and* ***specific risk assessment***

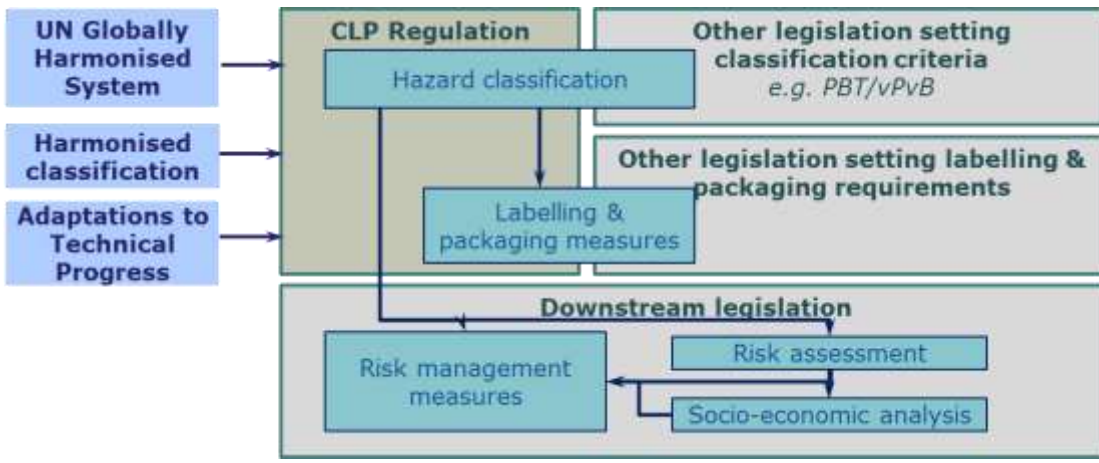
on authorisation of **plant protection** active ingredient and **products**

Community Strategy on **Endocrine Disrupters**

Regulation 528/2012 on authorisation of **biocidal** active ingredients and **products**

Regulation EC 1935/2004 **Food Contact Materials**

# General scheme



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## Direct application of risk management measures based on generic risk considerations


| Biocides | Toys | Cosmetics |
|---|---|---|
| *Public* | | |
| no | no* | no* |
| no | no* | no* |
| no | no* | no* |

* where contact with humans is avoided (PPPs and BPs), essential to prevent or control a serious danger to human health, animal health or the environment (BPs); allowed by the Scientific Committee on Consumer Safety (SCCS)



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## Hazards that trigger child-resistant fastening or tactile warnings

| Hazard Criteria | Child-resistant Fastenings | Tactile Warnings |
|---|---|---|
| Acute toxicity (category 1 to 3) | x | x |
| Acute toxicity (category 4) | | x |
| STOT single exposure (category 1) | x | x |
| STOT single exposure (category 2) | | x |
| STOT repeated exposure (category 1) | x | x |
| STOT repeated exposure (category 2) | | x |
| Skin corrosion (category 1A, 1B and 1C) | x | x |
| Respiratory sensitisation (category 1) | | x |
| Aspiration hazard (category 1)* | x | |
| Aspiration hazard (category 1) | x | x |
| Germ cell mutagenicity (category 2) | | x |
| Carcinogenicity (category 2) | | x |
| Reproductive toxicity (category 2) | | x |
| Flammable gases (category 1 and 2) | | x |
| Flammable liquids (category 1 and 2) | | x |
| Flammable solids (category 1 and 2) | | x |



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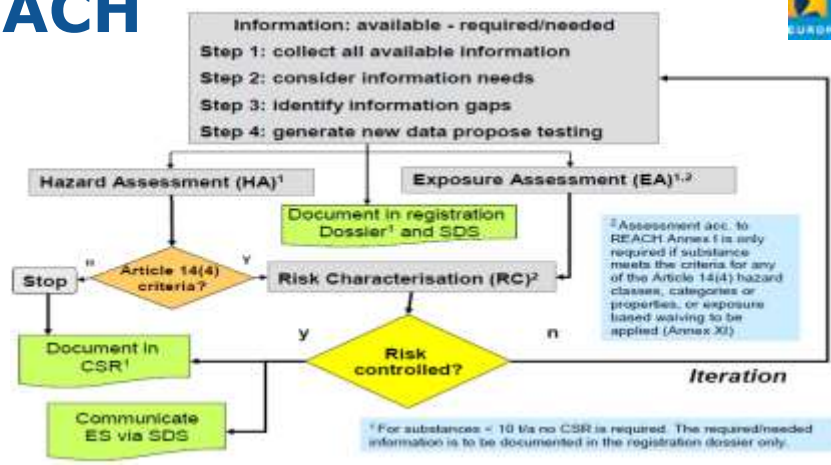
# REACH




**Guidance on information requirements and chemical safety assessment Ch. R.2, ver. 2.1 (2011)**

**CSR – Chemical Safety Report**
**SDS – Safety Data Sheet**
**ES – Exposure Scenarios**

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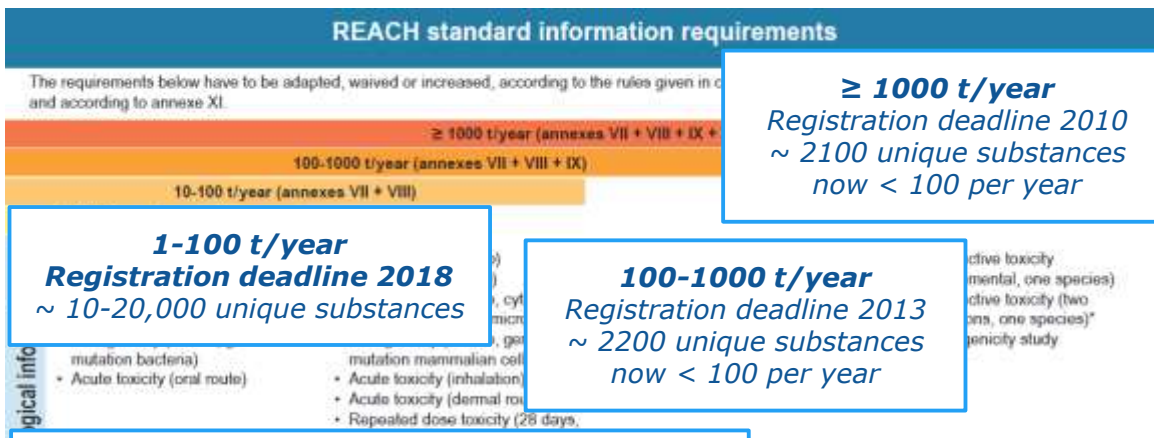


***≥ 1000 t/year***
*Registration deadline 2010*
*~ 2100 unique substances*
*now < 100 per year*

***1-100 t/year***
***Registration deadline 2018***
*~ 10-20,000 unique substances*

***100-1000 t/year***
*Registration deadline 2013*
*~ 2200 unique substances*
*now < 100 per year*

*On average:*
***1/3 mono-constituent***
***1/3 simple mixtures***
***1/3 UVCBs****
**Substance of Unknown or Variable composition, Complex reaction products or Biological materials*

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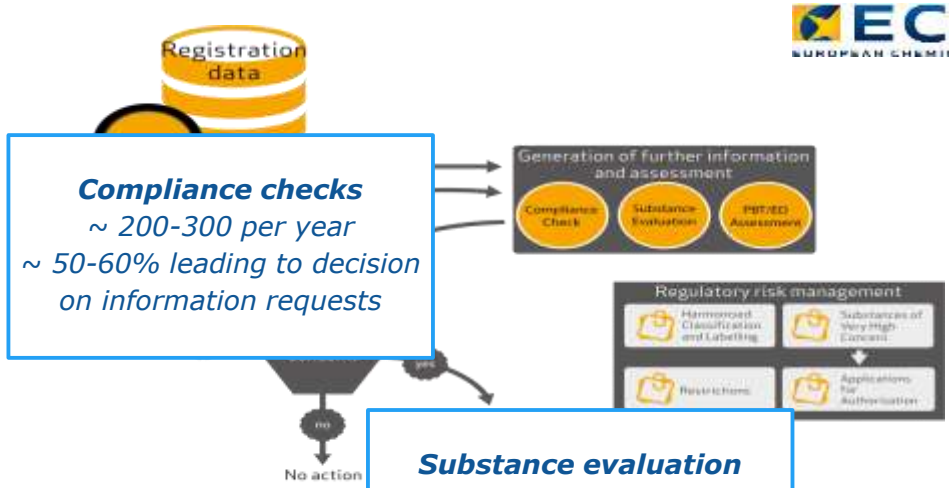
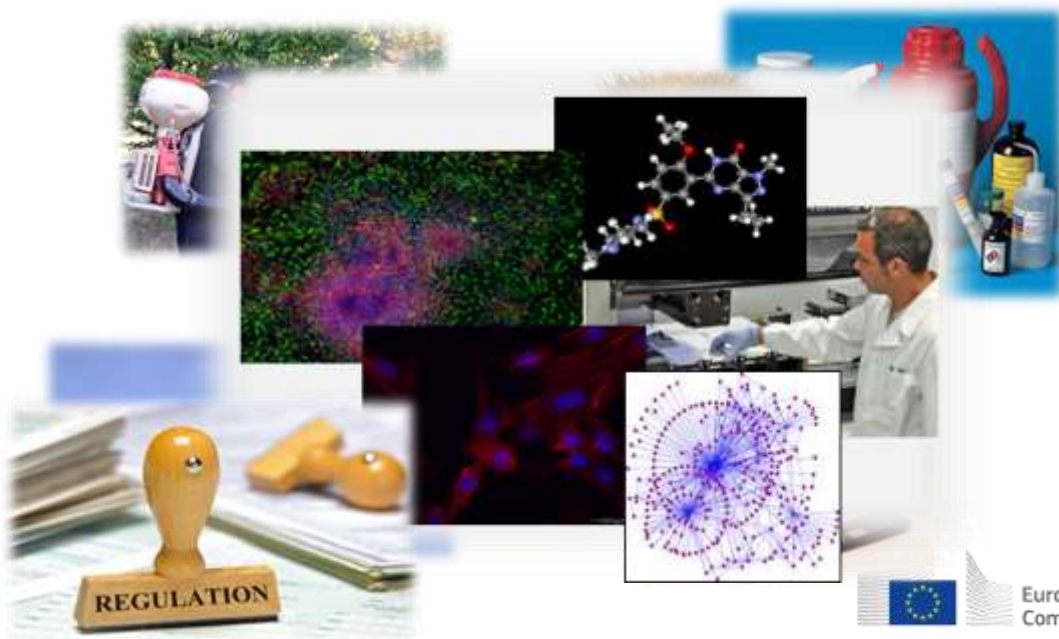



# REACH and Screening

# Alternative Test Methods

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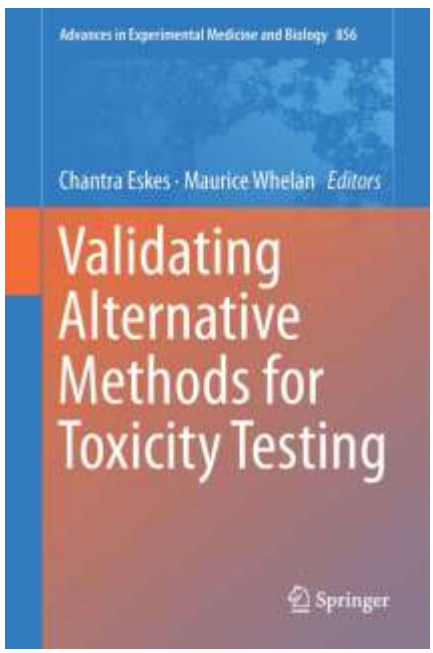


This book provides information on **best practices and new thinking** regarding the validation of alternative methods for toxicity testing. It covers the validation of **experimental and computational methods** and **integrated approaches to testing and assessment**. Validation strategies are discussed for **methods employing the latest technologies** such as tissue-on-a-chip systems, stem cells and transcriptomics, and for methods derived from **pathway-based concepts** in toxicology

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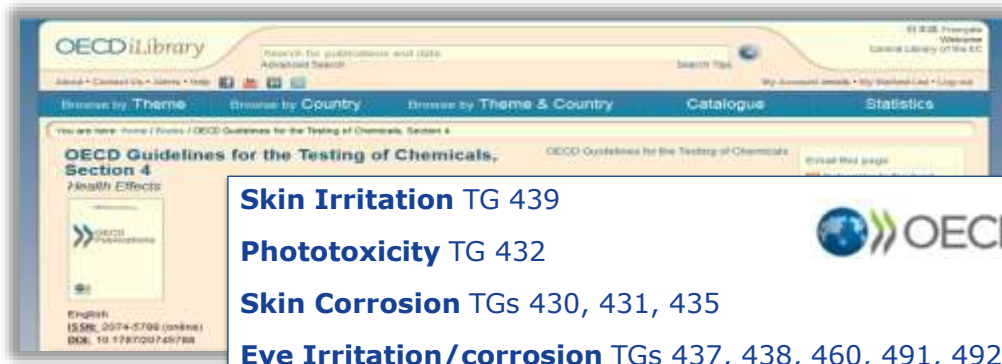


**Skin Irritation** TG 439

**Phototoxicity** TG 432

**Skin Corrosion** TGs 430, 431, 435

**Eye Irritation/corrosion** TGs 437, 438, 460, 491, 492

**Toxicokinetics** TG 428

**Genotoxicity** TGs 471, 473, 476, 487, 490

**Skin Sensitisation** TGs 442C, 442D, 442E

OECD

REACH

ICH

VICH

ISO International Organization for Standardization

cen

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# Validation in a nutshell

EURL ECVAM European Union Reference Laboratory for Alternatives to Animal Testing

*Attributes*

Scientifically sound principles

Well-defined

Applicable

Reproducible

Predictive

Transferable


**Reliable** and **Relevant** for a defined purpose

Independent peer review

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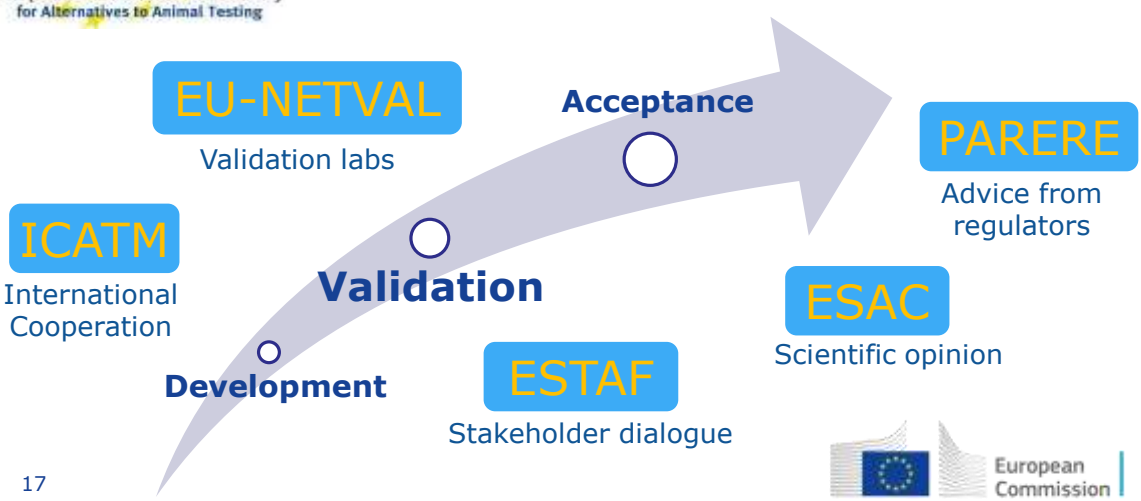
EURL ECVAM
European Union Reference Laboratory for Alternatives to Animal Testing

*Validation … assuring **sound science** to support **regulatory acceptance***





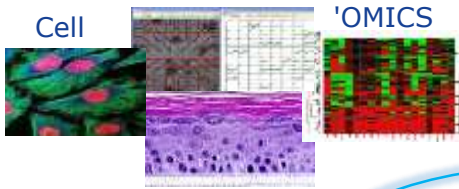

# TSAR

- **Just launched !!**
- Tracking from submission to acceptance
- Methods from EU, USA, Japan, Canada, Korea, and Brazil
- Access to method descriptions, key records and status comments

Organ-on-a-chip

Cell

'OMICS



3D tissues


**Reliability & Relevance ?**

Chemo-informatics
& Computational chemistry


Exposure modeling

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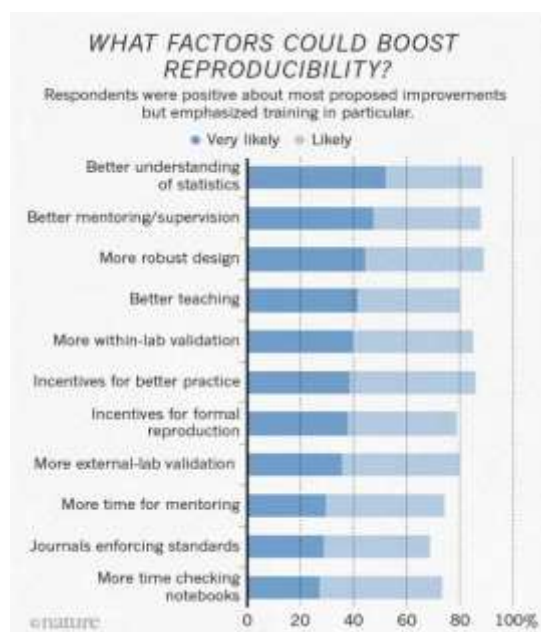
# Reliability


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)*



# Relevance

*Context*

*Benchmarks*



*Information*



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# IATA



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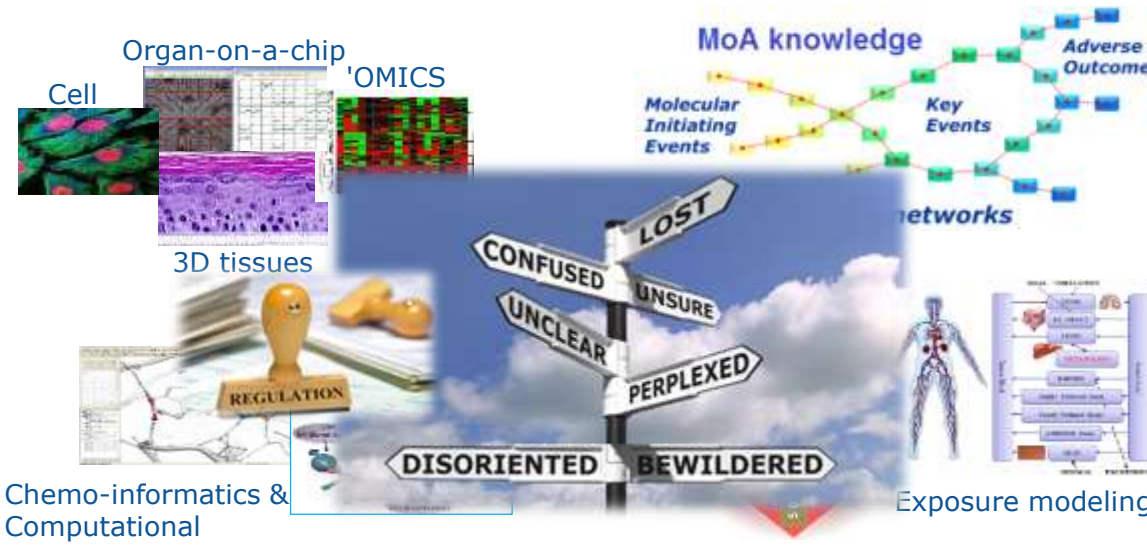




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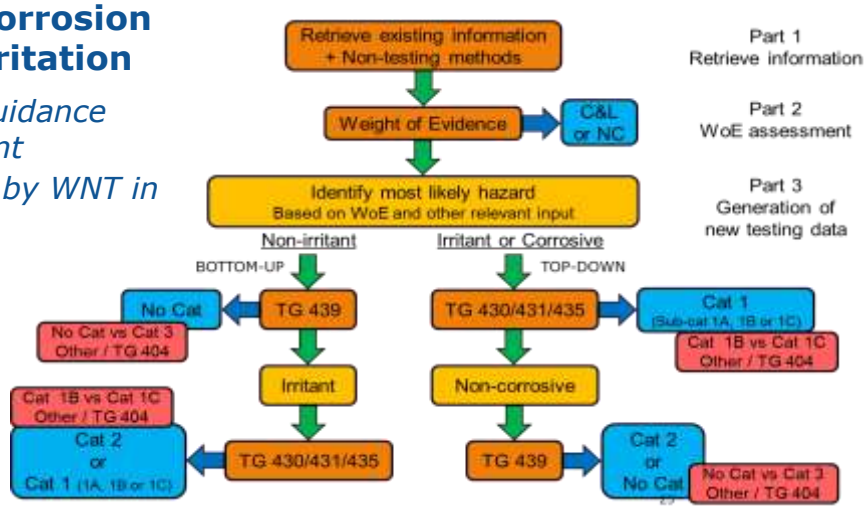
## Skin Corrosion and Irritation

*OECD Guidance Document adopted by WNT in 2014*



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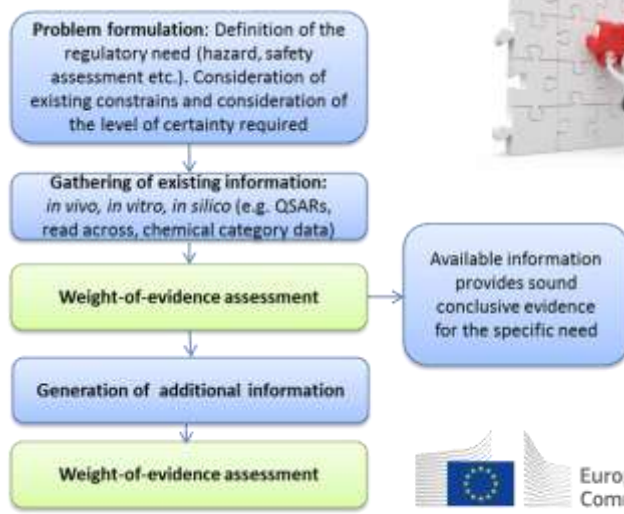
# Integrated Approach to Testing and Assessment (IATA)

*An IATA integrates and weights all relevant existing evidence and guides the targeted generation of new data where required to inform regulatory decisions*

***OECD Guidance Document 255***

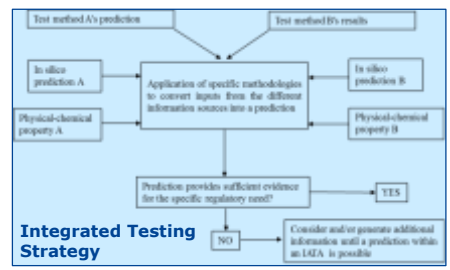


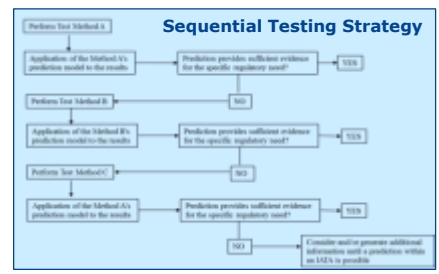



# Defined Approach

*Six defining principles:*

1. Defined toxicological endpoint
2. Defined regulatory purpose
3. Description of underlying rationale and mechanistic basis
4. Description of individual information sources used
5. Description of how information is processed and combined
6. Consideration of uncertainties



## Reporting Template for DA

OECD ENV/JM/MONO(2016)28

| | | |
|---|---|---|
| 1 | **Summary** | *concise overview of the approach* |
| 2 | **General information** | *identifier, date, authors, updates, references, proprietary aspects* |
| 3 | **Endpoint addressed** | *e.g. skin sensitisation* |
| 4 | **Purpose** | *e.g. screening, hazard assessment, potency prediction* |
| 5 | **Rationale underlying its construction** | *including reason for the choice of information sources and their linkage to known biological mechanisms (e.g. key events)* |
| 6 | **Brief description of individual information sources used** | *including response(s) measured and respective measure(s), detailed descriptions in the dedicated template* |
| 7 | **Process applied to derive the prediction** | *e.g. sequential testing strategies, regression models, 2 out of 3 WoE, scoring systems, machine learning approaches, Bayesian networks, etc…* |
| 8 | **Chemicals used to develop and test the approach** | *approach used for selection of training and test sets, relevant information on both sets: chemical names, composition, reference data (e.g. in vivo data), readouts, predictions* |
| 9 | **Limitations (and strengths) in the application of the approach** | *with regard to technical constrains or wrong predictions* |
| 10 | **Predictive capacity** | *misclassifications and unreliable predictions rationalised to the extent possible* |
| 11 | **Known uncertainties** | *how uncertainties related to approach, structure, information sources and benchmark data translate into prediction uncertainty* |


## Reporting Template for DA

| | | |
|---|---|---|
| 1 | **Summary** | *concise overview of the approach* |
| 2 | **General information** | *identifier, date, authors, updates, references, proprietary aspects* |
| 3 | | |
| 4 | | |
| 5 | | |
| 6 | | |
| 7 | | |
| 8 | | |
| 9 | **Limitations** | |
| 10 | **Predictive** | |
| 11 | **Known unc** | |





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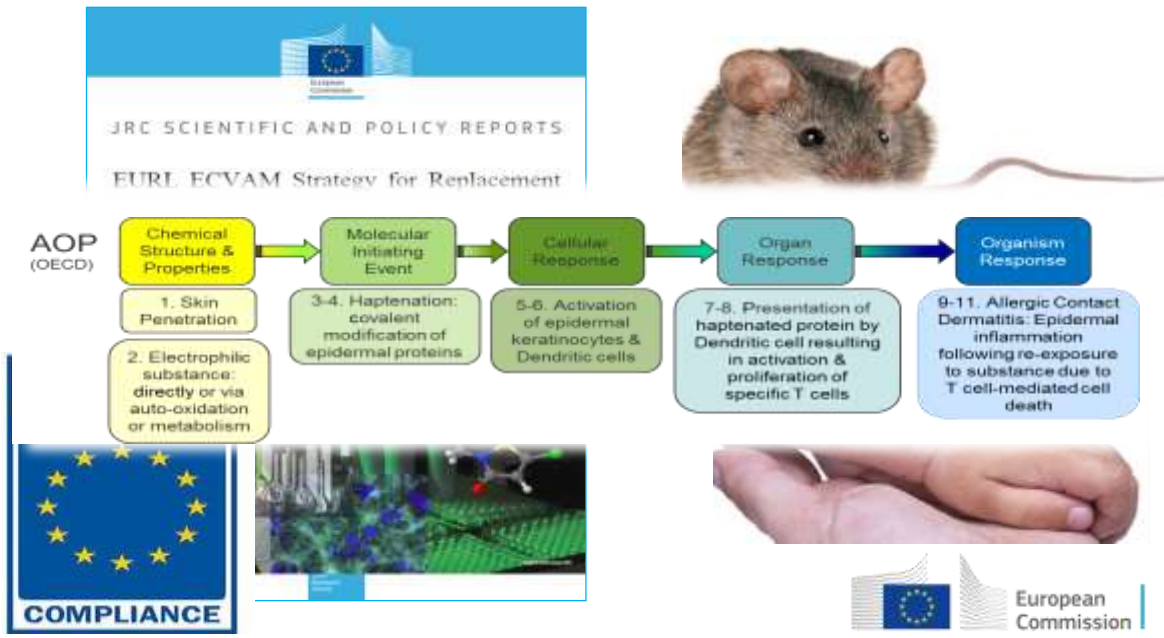
JRC SCIENTIFIC AND POLICY REPORTS
EURL ECVAM Strategy for Replacement
AOP
(OECD)
Chemical Structure & Properties
Molecular Initiating Event
Cellular Response
Organ Response
Organism Response
1. Skin Penetration
2. Electrophilic substance: directly or via auto-oxidation or metabolism
3-4. Haptenation: covalent modification of epidermal proteins
5-6. Activation of epidermal keratinocytes & Dendritic cells
7-8. Presentation of haptenated protein by Dendritic cell resulting in activation & proliferation of specific T cells
9-11. Allergic Contact Dermatitis: Epidermal inflammation following re-exposure to substance due to T cell-mediated cell death
COMPLIANCE
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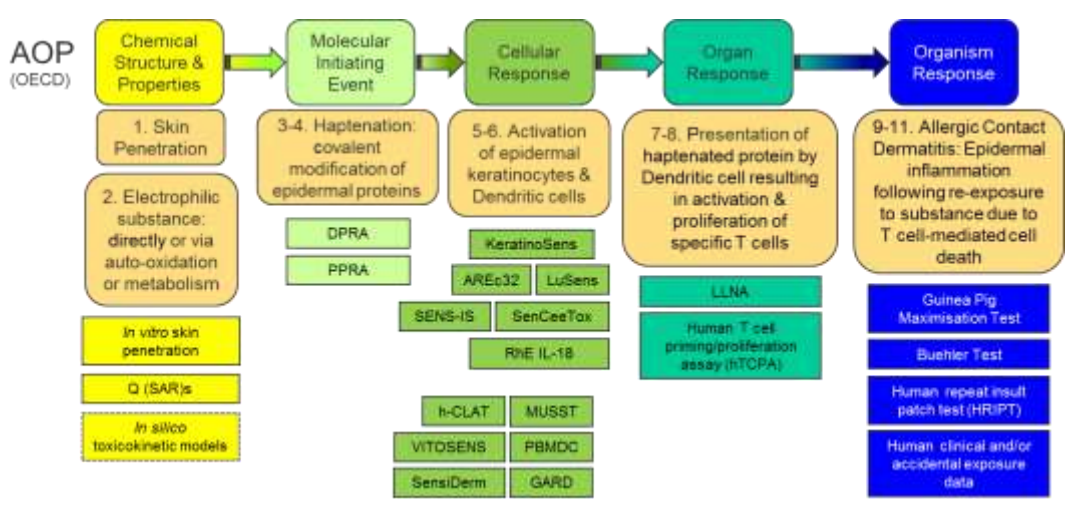
AOP
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Chemical Structure & Properties
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9-11. Allergic Contact Dermatitis: Epidermal inflammation following re-exposure to substance due to T cell-mediated cell death
In vitro skin penetration
Q (SAR)s
In silico toxicokinetic models
DPRA
PPRA
KeratinoSens
AREc32
LuSens
SENS-IS
SenCeeTox
RhE IL-18
h-CLAT
MUSST
VITOSENS
PBMDC
SensiDerm
GARD
LLNA
Human T-cell priming/proliferation assay (hTCPA)
Guinea Pig Maximisation Test
Buehler Test
Human repeat insult patch test (HRIPT)
Human clinical and/or accidental exposure data
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JOINT RESEARCH CENTRE

European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM)

European Commission > JRC > EURL ECVAM > Regulators adopt mechanistically-based non-animal test methods to assess the potential of chemicals to cause skin allergy

- Home
- Latest News
- About EURL ECVAM
- EURL ECVAM Strategy Papers
- EURL ECVAM Recommendations
- EURL ECVAM Status Reports
- Validation & regulatory acceptance
- Test Method Submission
- EU-NETVAL Validation Laboratories
- Laboratories & Research Areas
- Databases

# Regulators adopt mechanistically-based non-animal test methods to assess the potential of chemicals to cause skin allergy

Filed under: skin sensitisation, safety, EURL ECVAM, alternatives to animal testing

**EURL ECVAM validated methods adopted by the OECD paved the way for the revision of regulatory requirements for skin sensitisation under REACH**

On 20 April, the REACH Committee, comprising representatives of the EU Member States, adopted a revision to Annex VII of the REACH regulation which means that validated and accepted non-animal tests will become the default information requirement for assessing whether chemicals have the potential to cause skin sensitisation i.e. to induce an allergic response following skin contact. This will affect registrants who need to meet the 2018 REACH registration deadline for chemicals produced or imported in the range of 1-100 tonnes per year.

**News details**

Apr 26, 2016

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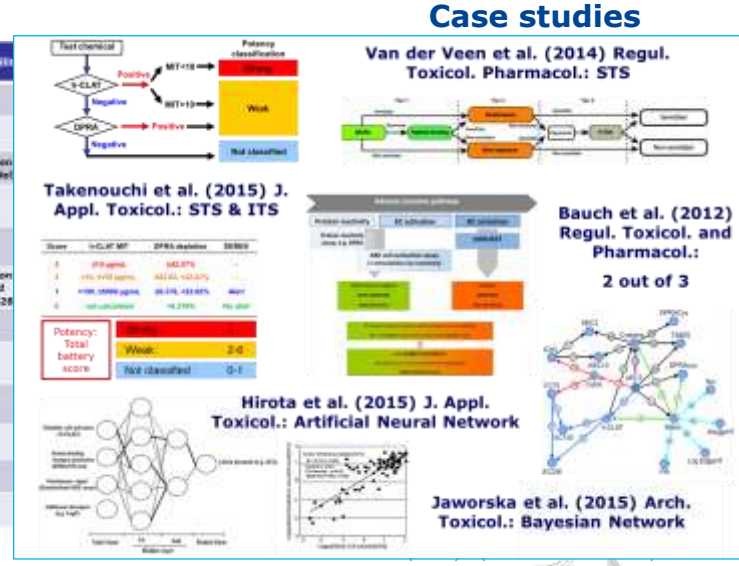
31

## Skin sensitisation

| | Case Study | Bioavailability |
|---|---|---|
| 1 | Sensitiser potency prediction Key event 3+2 (Givaudan) | |
| 2 | The artificial neural network model for predicting LLNA EC3 (Shiseido) | |
| 3 | ITS/DS for hazard and potency identification of skin sensitisers (P&G) | penetration (PBPK model |
| 4 | Tiered system for predicting sensitising potential and potency of a substance (STS) (Kao Corporation) | |
| 5 | Score-based battery system for predicting sensitising potential and potency of a substance (STS) (Kao Corporation) | |
| 6 | IATA for skin sensitisation risk assessment (Unilever) | penetration modified OECD TG428 |
| 7 | Weight of evidence in vitro ITS for skin hazard identification (BASF) | |
| 8 | STS for hazard identification of skin sensitisers (RIVM) | |
| 9 | IATA (Dupont) | |
| 10 | Decision strategy (L'Oréal) | |
| 11 | Integrated decision strategy for skin sensitisation hazard (NICEATM) | |
| 12 | Consensus decision tree model for skin sensitisation hazard prediction (EC JRC) | |

TG 442C : DPRA ; TG 442D : KeratinoSens™

## Case studies

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32

# REACH Annex VII revised legal text

| | | |
|---|---|---|
| '8.3. Skin sensitisation<br>Information allowing:<br>— a conclusion whether the substance is a skin sensitiser and whether it can be presumed to have the potential to produce significant sensitisation in humans (Cat. 1A), and<br>— risk assessment, where required. | The study(ies) t<br>if:<br>— the substan<br>— the substan<br>— the substan<br>water or m | conducted<br>6), or<br>ntact with |
| 8.3.1. Skin sensitisation, *in vitro/in chemico*<br>Information from *in vitro/in chemico* test method(s) recognised according to Article 13(3), addressing each of the following key events of skin sensitisation:<br>(a) molecular interaction with skin proteins;<br>(b) inflammatory response in keratinocytes;<br>(c) activation of dendritic cells. | The(se) test(s) d<br>— an *in vivo* st<br>— the availab<br>the substan<br>ment accor<br>If information<br>events in colu<br>cording to poi<br>be conducted. | icable for<br>sk assess-<br>f the key<br>sment ac-<br>need not |




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# Conventional toxicological endpoints

| Human Toxicity | | | Ecotoxicity and Fate |
|---|---|---|---|
| Acute Mammalian Toxicity (oral, dermal, inhalation) | Carcinogenicity | Mutagenicity/Genotoxicity | Persistence |
| Neurotoxicity | Systemic Toxicity/ Organ Effects<br>Repeated Dose Toxicity (oral, dermal, inhalation) | Respiratory Sensitization | Bioaccumulation |
| Skin Irritation and Corrosivity | Eye Irritation and Corrosivity | Endocrine Disruption | Chronic and Acute Aquatic Toxicity (on daphnia, algae and fish) |
| Reproductive and Developmental Toxicity | Skin Sensitization | | |

- *REACH and CLP (European Chemicals Agency, 2012)*
- *SIDS Manual for the Assessment of Chemicals (OECD, 2011)*
- *World Health Organization - Human Health Risk Assessment (WHO, 2010)*
- *The GHS (United Nations, 2009)*
- *Assessment Criteria for Hazard Evaluation version 2.0 (US EPA DfE, 2011)*
- *Guide on Sustainable Chemicals (UBA, 2011)*
- *Washington State Department of Ecology Quick Chemical Assessment Tool (QCAT)*
- *NSF/GCI/ANSI 355 – 2011, Greener Chemicals and Processes Standard (ANSI, 2013)*

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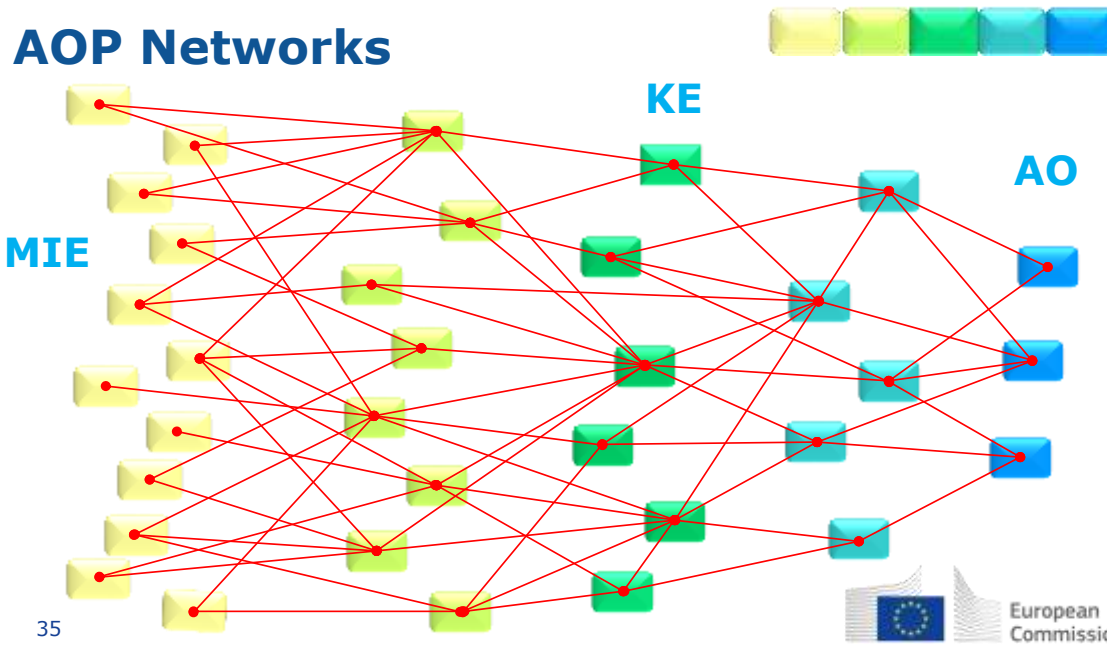
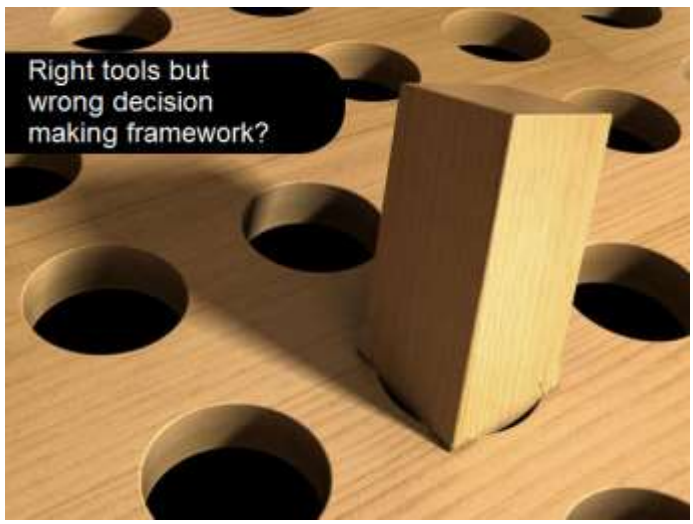
# AOP Networks








***What should we really be trying to predict?***

***What predictions can provide protection?***

# Uncertainty





Extrapolating from early to late effect

Extrapolating across dosing duration

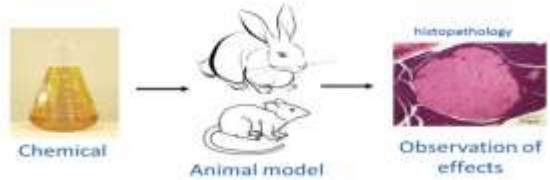
Extrapolating across dosing patterns

## Conventional Toxicology

Determination of a PoD

Extrapolating to low-effect levels

Estimating Intra-species variability




Extrapolating across exposure metrics

Extrapolating across agents

## Sources of 'familiar' uncertainty

Inter-species extrapolation

Estimating the impact of missing studies

Extrapolating from *in vitro* or *in chemico* to *in vivo* data

**WHO-IPCS (2014) Guidance document on evaluating and expressing uncertainty**

SCIENTIFIC OPINION

**Guidance on Uncertainty in EFSA Scientific Assessment**

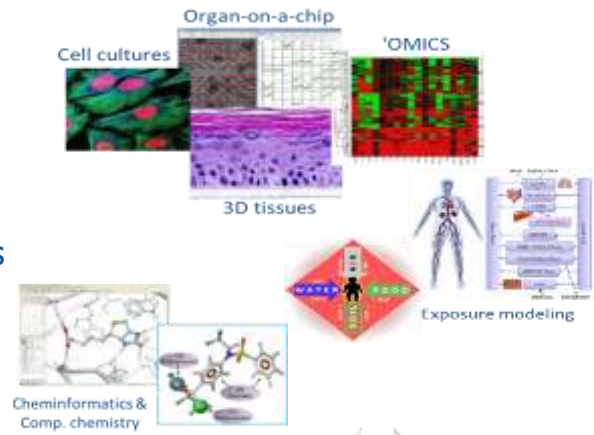
**EFSA Scientific Committee**[1, 2]
European Food Safety Authority (EFSA), Parma, Italy

DRAFT

EFSA Journal

# Uncertainty of inputs

- Ambiguity
- Measurement uncertainty
- Sampling uncertainty
- Assumptions incl. default values
- Extrapolation uncertainty
- Distribution uncertainty
- Other uncertainties

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SCIENTIFIC OPINION

**Guidance on Uncertainty in EFSA Scientific Assessment**

**EFSA Scientific Committee**[1, 2]
European Food Safety Authority (EFSA), Parma, Italy

DRAFT

EFSA Journal

# Uncertainty when combining inputs

- Ambiguity and excluded factors
- Relationship between components
- Distribution uncertainty
- Structure of the assessment
- Comparisons with independent data
- Dependency between uncertainties


$$f(x|n) = \binom{n}{x} p^x q^{n-x},\ 0 \le x \le n$$
$$f(x,n) = \binom{n}{x} p^x q^{n-x} a_n,\ 0 \le x \le n$$
$$m(x) = \sum_{k=x}^{m} \binom{n}{x} p^x q^{n-x} a_n = p^x \sum_{k=x}^{m} \binom{n}{x} q^{n-x} a_n =$$
$$p^x \sum_{k=x}^{m} \binom{n+x}{x} q^k a_{k+x}$$
$$f(n|x) = \frac{f(x,n)}{m(x)} = \frac{\binom{n}{x} p^x q^{n-x} a_n}{p^x \sum_{k=x}^{m} \binom{n+x}{x} q^k a_{k+x}} = \frac{\binom{n}{x} q^{n-x} a_n}{\sum_{k=x}^{m} \binom{n+x}{x} q^k a_{k+x}}$$

Categorisation / Read-across

Testing Strategies (ITS / STS)

Weight of Evidence

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# Scientific Credibility



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## Scientific Credibility

The willingness of others to use predictions to inform their decisions*

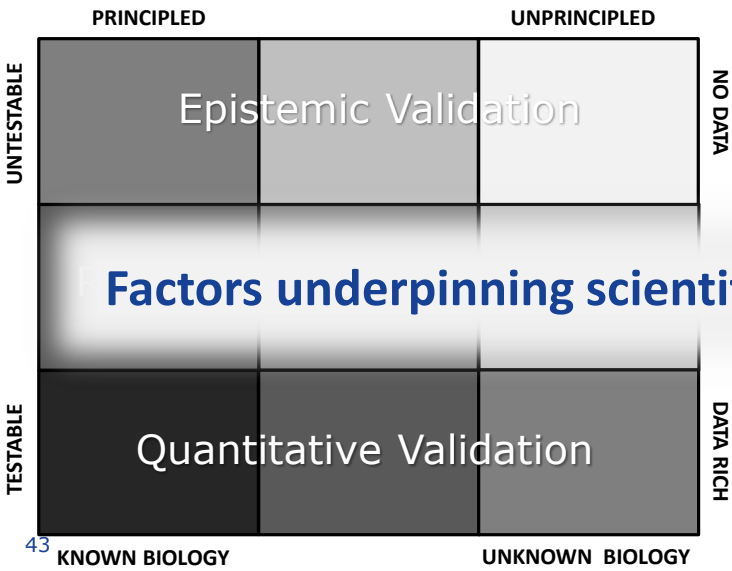
It is established through a process of **social epistemology** to develop a *shared* knowledge and understanding between key actors (i.e. developers, end-users, assessors, regulators, …) through (personal) interactions

*Schruben LW, Establishing credibility of simulations, *Simulation*, 34:101-105, 1980.



European Commission

# Credibility Matrix for computational biology




Patterson & Whelan;

"A framework to establish credibility of computational models in biology",

*ics*
*gy*
129C (2017) pp. 13-19



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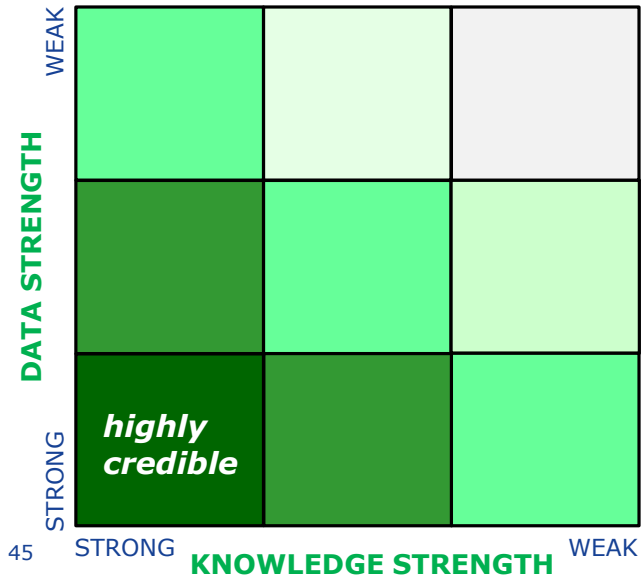
| | |
|---|---|
| **Confirmed assumptions** | ➢ Identify assumptions underpinning approach & their limitations |
| | ➢ Collate observational evidence to justify each assumption |
| **Qualitative concordance** | ➢ Assess extent to which predicted behavioural trends match observations |
| **Quantitative concordance** | ➢ Quantify how predictivity with respect to target effect |
| **Explanatory power** | ➢ Explain observed phenomena and behaviour [effects] using predictions |
| | ➢ Explain situations & effects other than those on which approach is based |
| **Internal coherence** | ➢ Demonstrate approach predicts already known result (calibration) |
| | ➢ Demonstrate perturbation of input parameters produces expected result |
| | ➢ Demonstrate predicted behaviour disappears in appropriate circumstances |
| | ➢ Demonstrate predictions unchanged by elimination of all sources of error |
| **External consistency** | ➢ Predict similar outcomes with an alternative approach[es] |
| | ➢ Assess reproducibility of approach in different environments |
| **Simplicity** | ➢ Demonstrate appropriate degree of complexity by removal of each core assumption producing a significant change in prediction |
| | ➢ Build narrative with appropriate detail that is both precise and concise |

## Credibility Matrix for predictive approaches




Patterson & Whelan;

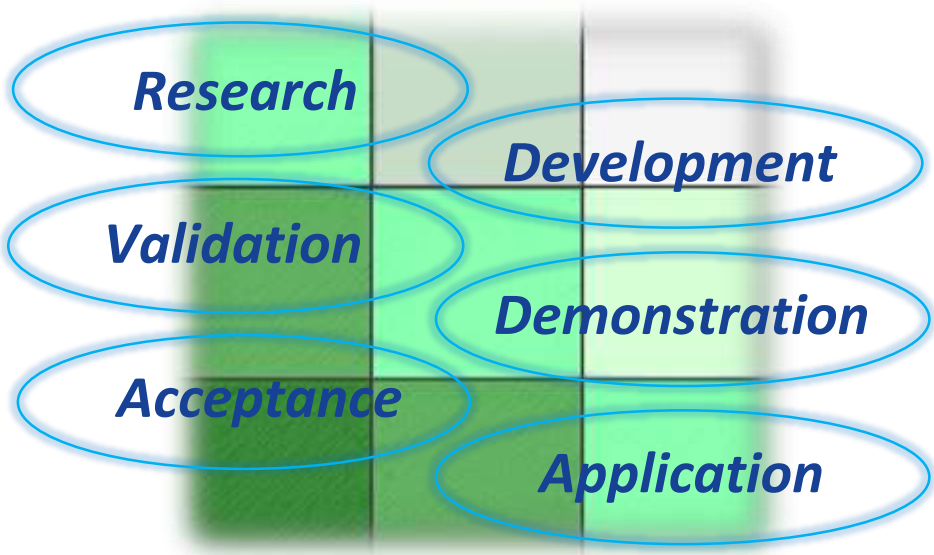
"Establishing the credibility of predictive toxicology approaches intended for regulatory purposes"

*In preparation (nearly there!)*

Strength:
Availability, applicability and dependability

Knowledge:
Acquaintance with facts, truths and principles






***Social Epistemology***

*.. process is as important as result!*

# In summary

- Clear shift in emphasis from individual methods to IATA.
- Transparency, clarity and thoroughness in describing IATA are fundamental to facilitate evaluation and acceptance.
- Key question - how do we strike the right balance regarding *flexibility (IATA) versus prescriptiveness (DA)* to embrace new science but address the practicalities of regulatory implementation and industry needs.
- Validation needs to keep pace with innovation.
- Establishing credibility and confidence relies on constructive engagement throughout the process





# Stay in touch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