



The European Union Reference Laboratory for Alternatives to Animal Testing

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Institute for Health and Consumer Protection
DG Joint Research Centre

AIMBE/NIH Workshop
March 2014

Talk Outline

Validation of new tools ...



Overview of EURL ECVAM



Validation in a regulatory context

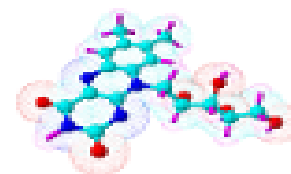


Validation in a research context

Our EU Policy focus

Regulation of Chemicals and the Protection of Animals

- Animal-free methodology to assess the hazard posed to consumers, workers and the environment
- Industrial chemicals, cosmetic ingredients, food constituents, pesticides, biocides and drugs
- Advancing safety assessment science to support regulatory decision making



DG JRC - Institute for Health and Consumer Protection

Systems Toxicology Unit / EURL ECVAM

75 staff

Groups

Assay
Validation

Predictive
Toxicology

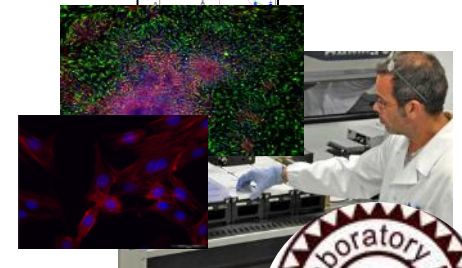
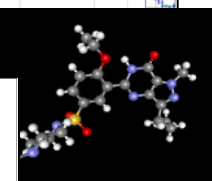
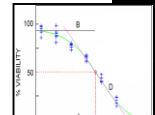
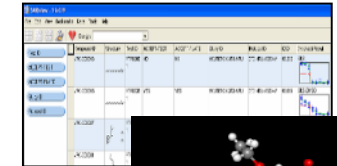
Toxicity
Pathways

Facilities

In Vitro
GLP

HTS
HCS

Projects



Integrated approaches to testing and assessment

Standardisation and international harmonisation of alternative methods

Combined exposures and chemical mixtures

Endocrine disruptors

Information systems for safety assessment and alternative methods

DIRECTIVE 2010/63/EU OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL
of 22 September 2010
on the protection of animals used for scientific purposes

"While it is desirable to replace the use of live animals in procedures by other methods not entailing the use of live animals, the use of live animals continues to be necessary to protect human and animal health and the environment. However, **this Directive represents an important step towards achieving the final goal of full replacement of procedures on live animals for scientific and educational purposes as soon as it is scientifically possible** to do so. To that end, it seeks to facilitate and promote the advancement of alternative approaches."

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

The European Union Reference Laboratory for Alternatives to Animal Testing

Key responsibilities*

- Coordinate and promote development and use
- Coordinate validation at Union level
- Information exchange on development
- Databases and information systems
- Promote dialogue between legislators, regulators, and stakeholders



*Article 48 of the Directive, Annex VII



European Commission

Browser address bar: <http://ihcp.jrc.ec.europa.eu>

Navigation: European Commission > JRC > IHCP

JOINT RESEARCH CENTRE

Institute for Health and Consumer Protection (IHCP)

European Commission > JRC > IHCP > Our Reference Centres and Laboratories > EURL ECVAM (European Union Reference Laboratory for Alternatives to Animal Testing)

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

- Latest News
- About EURL ECVAM
- New to EURL ECVAM?
- EURL ECVAM's Validation Process
- Test Method Submission
- Validation & regulatory acceptance
- EURL ECVAM Recommendations
- EURL ECVAM Strategy Papers
- Advisory and Consultation Bodies
- EU-NETVAL (European Union Network of Laboratories for the Validation of Alternative Methods)
- Laboratories & Research
- International collaboration
- Databases
- Archive of Publications
- Open Calls
- Contact

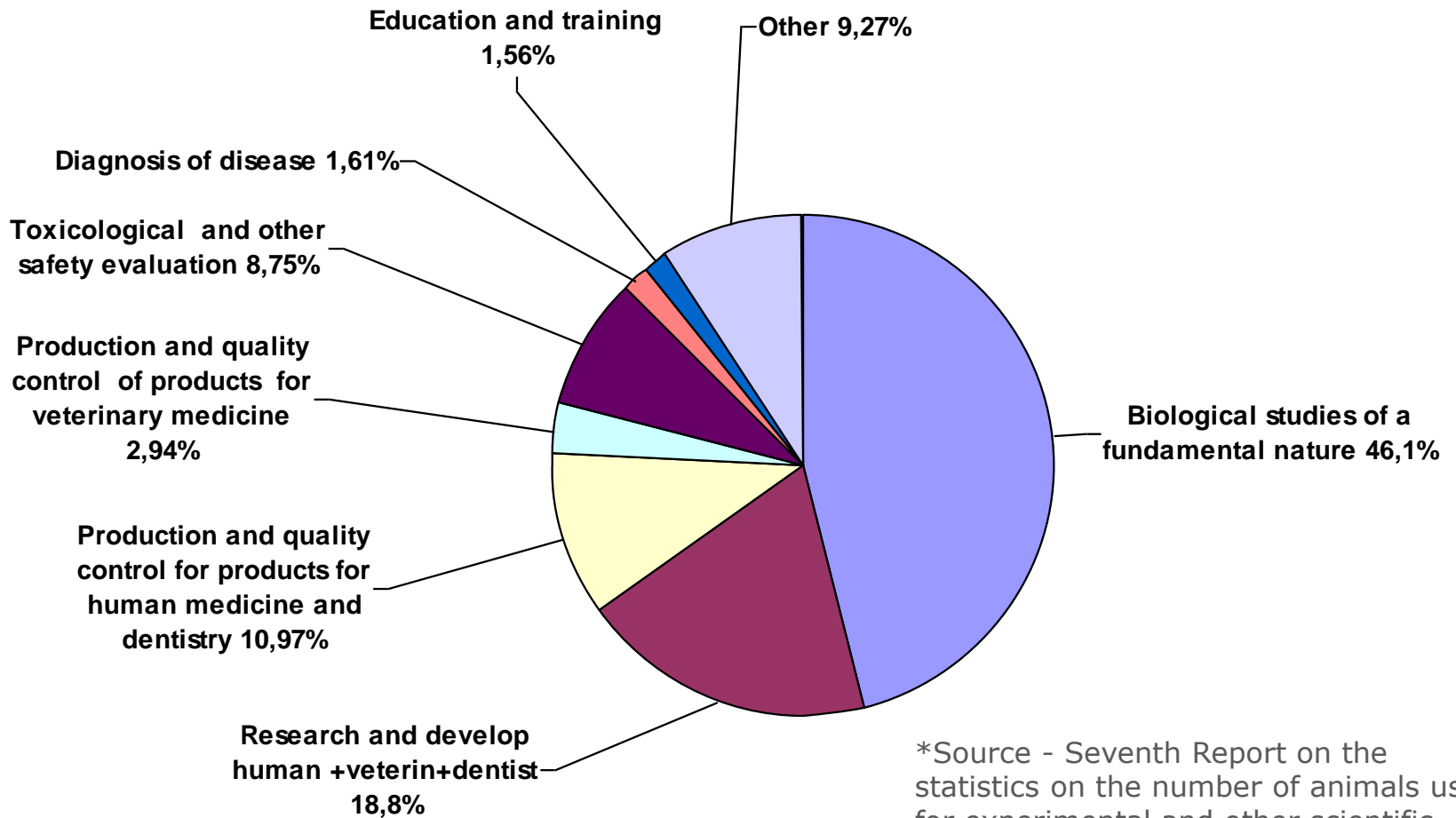
EURL ECVAM's latest tweets

Subscribe to an always-updated feed of these search terms:

- [Alternatives to animal testing: EURL ECVAM publishes its Recommendation on the KeratinoSens™ assay for skin sensitisation testing](#)
KeratinoSens™ may prove a useful component of integrated approaches for skin sensitisation hazard assessment, and may also be able to contribute to the ...
Alternatives to Animal Testing | Feb 19, 2014
- [SEURAT-1 meeting showcases new results and challenges in alternative methods to animal testing](#)
Repeated dose systemic toxicity testing of chemicals : highlights from the 4th Annual Meeting of SEURAT-1 (Barcelona, 5-6 February 2014)
Alternatives to Animal Testing | Feb 18, 2014
- [SEURAT-1 Annual Meeting \(5-6 Feb 2014\): alternative methods for repeated dose systemic toxicity testing of chemicals](#)
Major scientific achievements and new challenges
Alternatives to Animal Testing | Jan 30, 2014
- [Striving for 21st century toxicology – exploiting mechanistic understanding to further alternative methods](#)
Report on how Toxicity Pathway concepts can be applied in chemical safety assessment
Alternatives to Animal Testing | Jan 24, 2014
- [Transatlantic cooperation to advance in vitro methods in safety science using high throughput screening technology](#)
JRC's EURL ECVAM and NIH's NCATS meet to devise a work plan for collaboration
Alternatives to Animal Testing | Jan 21, 2014
- [Watch EURL ECVAM's new video: Advancing safety assessment without animals](#)
EURL ECVAM scientists inform you on their latest activities and achievements
Alternatives to Animal Testing | Jan 09, 2014
- [How to reduce animal use in genotoxicity testing : EURL ECVAM releases its strategy](#)
Satisfying regulatory safety assessment with alternative methods
Alternatives to animal testing | Dec 18, 2013
- [Alternatives to animal testing: EURL ECVAM publishes its Recommendation on a Cell Transformation Assay for carcinogenicity testing based on the Bhas 42 cell line](#)
In its Recommendation, EURL ECVAM summarises key performance parameters of this in vitro method and its use within integrated approaches to assessing the ...
Alternatives to Animal Testing | Dec 17, 2013

Joint Research Centre

Statistics on the purposes of the procedures carried out in the 27 Member States in 2011*



*Source - Seventh Report on the statistics on the number of animals used for experimental and other scientific purposes in the EU MS (in preparation)

ECVAM DataBase service on Alternative Methods

DB-ALM



DB-ALM - Windows Internet Explorer
http://ecvam-dbalm.jrc.ec.europa.eu

JOINT RESEARCH CENTRE
EURL ECVAM DataBase service on ALternative Methods to animal experimentation (DB-ALM)

European Commission > JRC > IHCP > EURL ECVAM > DB-ALM > Methods Search

METHODS SEARCH | QSAR MODELS SEARCH | BIBLIOGRAPHIC SEARCH | COMPREHENSIVE LISTINGS | GUIDED SEARCH | FREE TEXT SEARCH

Method Summaries

Topics: Choose one or more topics >>

Models & Strategies: Choose one or more model types >>

Method/Assay name: Choose one or more method names >> [free text search field]

Experimental system: [free text search field]

Endpoints & Endpoint measurements:

- Corneal epithelial integrity
- Corneal hydration
- Corneal opacity
- Corneal permeability

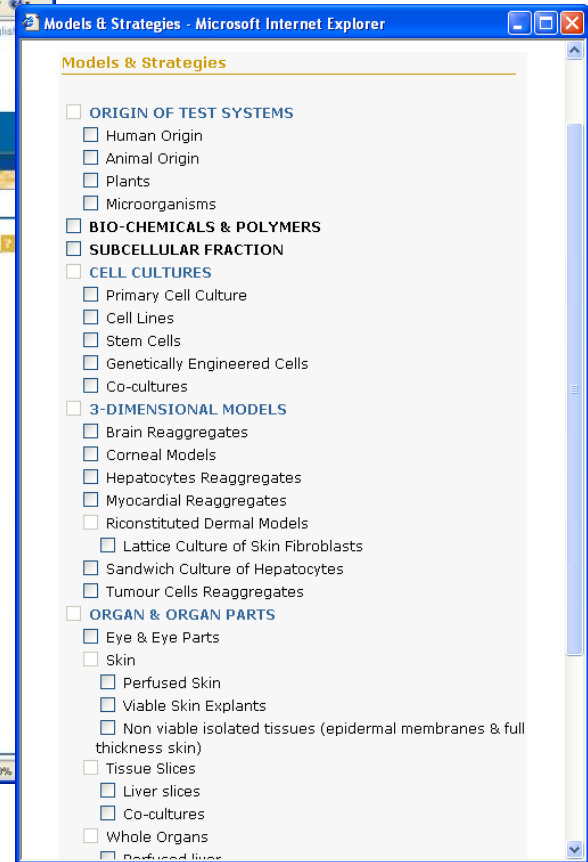
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Method status:

- All
- Participation in evaluation studies
- Participation in EU integrated projects
- Participation in validation studies
- Scientifically validated
- National standard
- Regulatory acceptance / Guideline compliance

[Search] [Clear]

query summary



Models & Strategies - Microsoft Internet Explorer

Models & Strategies

- ORIGIN OF TEST SYSTEMS
 - Human Origin
 - Animal Origin
 - Plants
 - Microorganisms
- BIO-CHEMICALS & POLYMERS
- SUBCELLULAR FRACTION
- CELL CULTURES
 - Primary Cell Culture
 - Cell Lines
 - Stem Cells
 - Genetically Engineered Cells
 - Co-cultures
- 3-DIMENSIONAL MODELS
 - Brain Reaggregates
 - Corneal Models
 - Hepatocytes Reaggregates
 - Myocardial Reaggregates
 - Riconstituted Dermal Models
 - Lattice Culture of Skin Fibroblasts
 - Sandwich Culture of Hepatocytes
 - Tumour Cells Reaggregates
- ORGAN & ORGAN PARTS
 - Eye & Eye Parts
 - Skin
 - Perfused Skin
 - Viable Skin Explants
 - Non viable isolated tissues (epidermal membranes & full thickness skin)
 - Tissue Slices
 - Liver slices
 - Co-cultures
 - Whole Organs
 - Perfused liver

<http://ecvam-dbalm.jrc.ec.europa.eu>

Promoting a broader use of alternatives

EURL ECVAM Search Guide

- Search principles & procedures, terms and guidance
- 7-step check list to ensure comprehensive searches
- Inventory of key resources



**Support to project authorisation
process for animal experiments**

(Directive 2010/63/EU)

Free from:

<http://bookshop.europa.eu>

Top 4!



EURL ECVAM interactions with regulators, stakeholders and scientific advisors

Dialogue with regulators

PARERE

Preliminary
Assessment of
Regulatory Relevance
(MS, COM, Agencies)

Stakeholder
dialogue

ESTAF

ECVAM Stakeholder
Forum

Scientific advice

ESAC

ECVAM Scientific
Advisory Committee



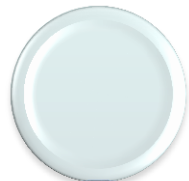
ICATM

International Cooperation on Alternative Test Methods

- Founded in 2009 by JaCVAM (Japan), NICEATM-ICCVAM (USA), EURL ECVAM (EU), Health Canada, and KoCVAM (Republic of Korea). Recent interest from BraCVAM (Brazil). Contacts with China.
- Working together at every step of the process: Evaluation of Promising Methods; Design and Execution of Validation Studies, Peer Review, Recommendations, Development of Int. Guidance and Guidelines, Dissemination).
- Latest Meeting in November 2013 led to a number of solid outcomes and actions.

Talk Outline

Validation of new tools ...



Overview of EURL ECVAM

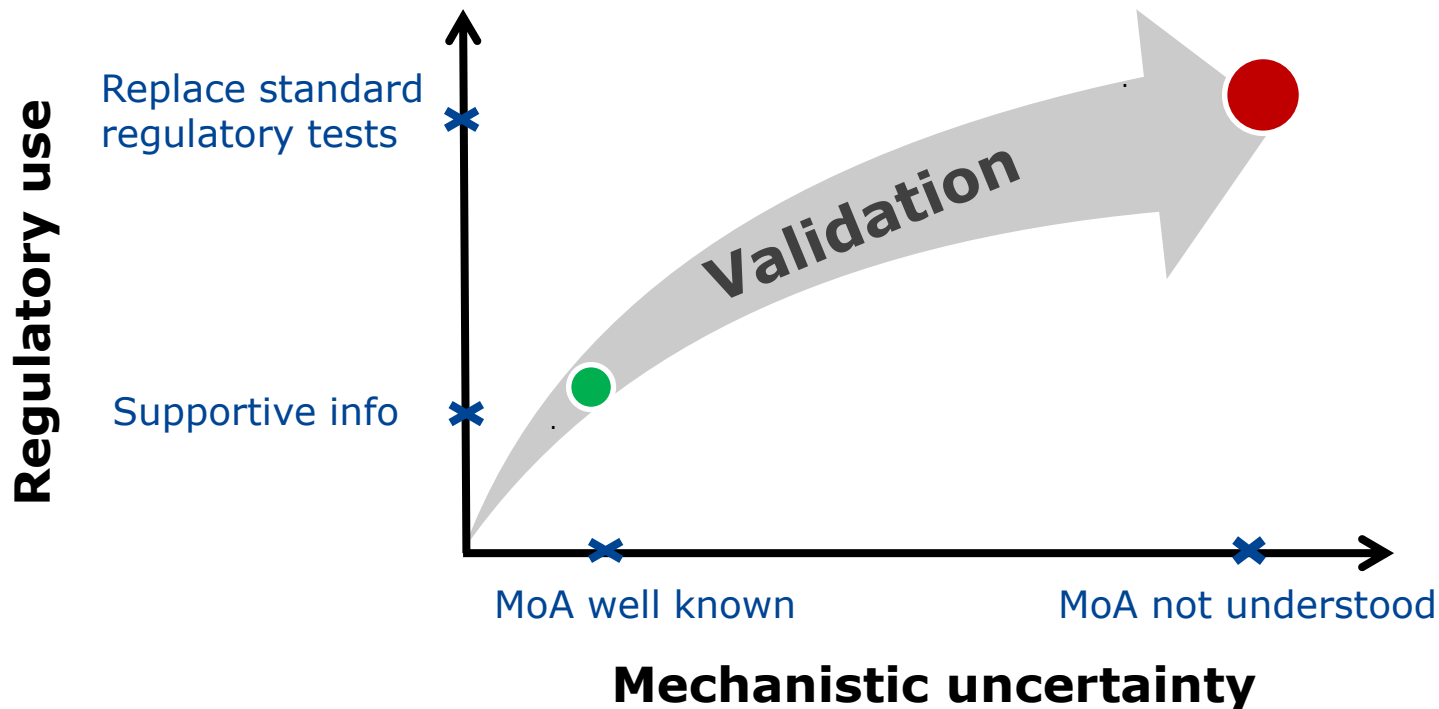


Validation in a regulatory context



Validation in a research context

Approaches to validation depend on context



3Ps of Validation: Principles, Purpose and Process

Consultation

ICATM: Exchange info. on promising methods. Decide how to collaborate

PARERE & ESTAF:
Initial assessment of relevance and utility

ICATM: Review aspects of VS. Propose VMG members and observers

Ad hoc consultations:
On study design and related information

ICATM:
Proposal of experts & observers for ESAC WGs

ICATM:
Input to strengthen claims and achieve harmonisation/impact

PARERE, ESTAF, ICATM, Public, Test submitter

Validation Work Flow

1. Assessment of submitted method
Evaluate status and prioritise

2. Undertake Validation Study
Characterise reliability and relevance

3. Independent Peer Review
ESAC assessment of study and outcome

4. ECVAM Recommendation
Propose uses and further steps

Records

Test Submission

Evaluation report

Validation Study Plan

Validation Report

ESAC Request

ESAC report & Opinion

EURL ECVAM Recommendation With Annexes

Recorded comments

Submitter Response

JOINT RESEARCH CENTRE

Institute for Health and Consumer Protection (IHCP)

> Our Reference Centres at http://ihcp.jrc.ec.europa.eu/our_labs/eurl-ecvam/eurl-ecvam-recommendations

EURL ECVAM Recommendations

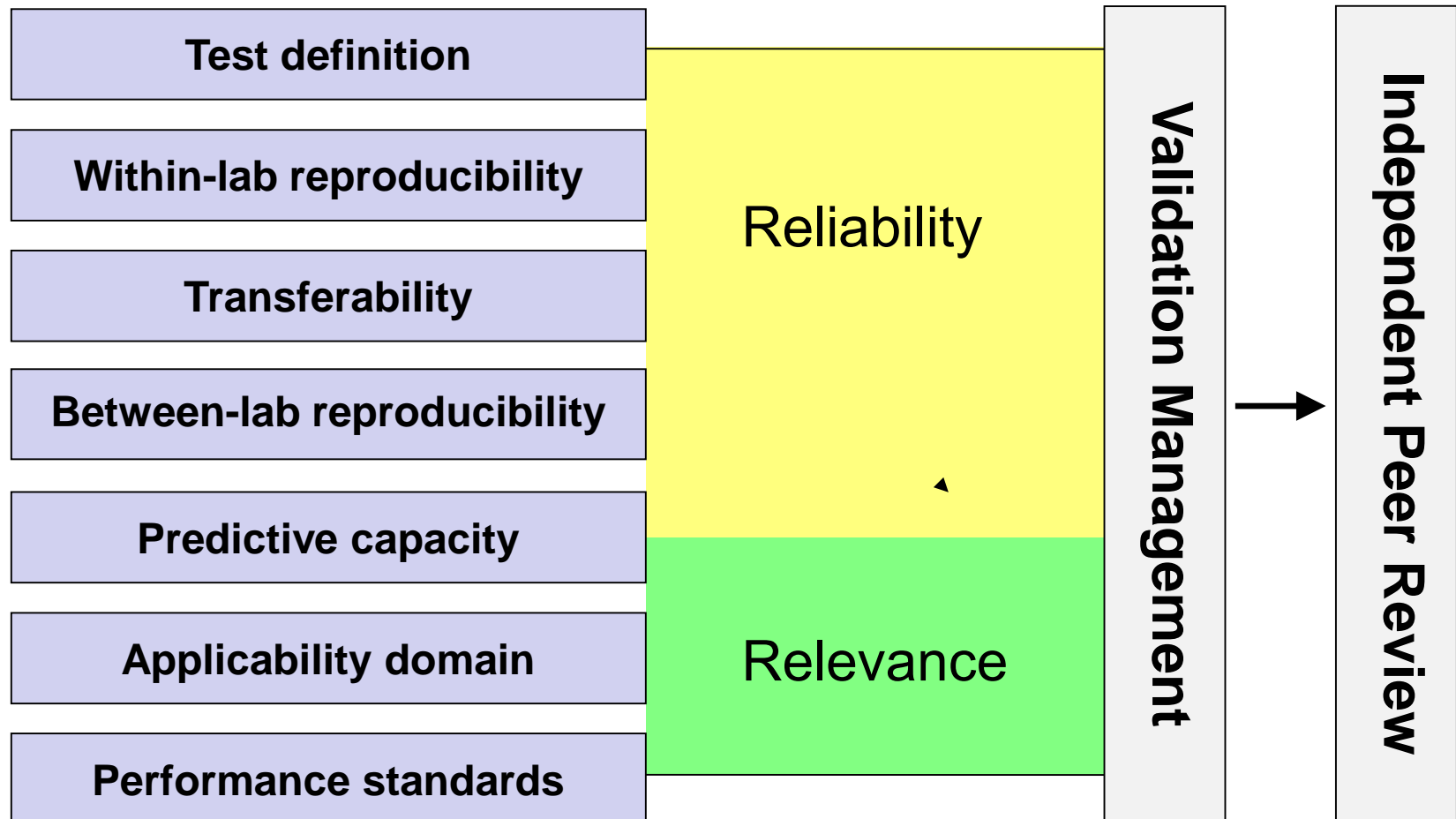
The aim of an EURL ECVAM Recommendation is to provide EURL ECVAM views on the validity of the test method in question, its regulatory applicability, limitations and proper scientific use of the test methods, and to suggest possible follow-up actions to address knowledge gaps.

During the development of its Recommendation EURL ECVAM consults with its consultation body for Preliminary Assessment of Regulatory Applicability (PARA), its EURL ECVAM Stakeholder Forum (ESTAF). Moreover, EURL ECVAM consults with other Commission services and its international partners of the International Collaboration on Alternative Test Methods (ICATM). Moreover, before finalising its recommendations, EURL ECVAM consults the general public and, if applicable, from the test method submitter.

Health / environmental endpoint	Recommendation
Skin Sensitisation	KeratinoSens™ assay for Skin Sensitisation testing
Carcinogenicity	Cell Transformation Assay for carcinogenicity testing based on the Bhas 42 cell line
Skin Sensitisation	Direct Peptide Reactivity Assay (DPRA)
Acute Oral Toxicity	3T3 NRU Assay for Supporting the Identification of Substances Not Requiring Classification for Acute Oral Toxicity
Carcinogenicity	Three Cell Transformation Assays for in vitro Carcinogenicity Testing



Validation – a modular approach





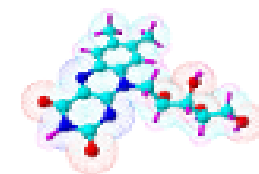
Validation towards Standards

Reporting STD: For *describing a method* and the *results obtained* from it for a particular test item.

Physical STD: Reference chemicals with clearly defined structural, physiochemical, mechanistic, toxicological and toxicokinetic properties.

Methodological STD: Protocols to characterise a method using physical STD to derive information on *basic performance* and *application-specific* performance.

*Underway – Project to develop STD for AR Transactivation Assays
Coming soon! Project to develop STD for TK/Clearance methods
(inc. web-based survey of methods)*



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

→ Latest News

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→ Validation & regulatory acceptance

→ EURL ECVAM Recommendations

→ EURL ECVAM Strategy Papers

→ Advisory and Consultation Bodies

→ EU-NETVAL (European Union Network of Laboratories for the Validation of Alternative Methods)

→ Laboratories & Research

→ International collaboration

EU-NETVAL (European Union Network of Laboratories for the Validation of Alternative Methods)

EU-NETVAL's mission is to provide support for EURL ECVAM validation studies that serve to assess the reliability and relevance of alternative methods that have a potential to replace, reduce, or refine the use of animals for scientific purposes.

EU-NETVAL will be coordinated and sustained by the EURL ECVAM, in close collaboration with [Directorate-General for Environment](#) (to ensure interactions with the National Contact Points for [implementation of Directive 2010/63/EU on the protection of animals used for scientific purposes](#)).

EU-NETVAL was set up by EURL ECVAM in response to the provision of [Directive 2010/63/EU](#) on the protection of animals used for scientific purposes which requests that EU Member States assist the European Commission in the validation of alternative methods. Currently there are a total of **26 members of EU-NETVAL**, selected against pre-defined [eligibility criteria](#) (including **25 test facilities** from EU Member States plus the European Commission's own *in vitro* [GLP test facility operated by EURL ECVAM](#), which coordinates the network) and approved by the National Contact Points.

The [Terms of Reference for EU-NETVAL](#) detail the legislative anchor, the establishment of the network and the maintenance of its membership, tasks of network members and of EURL ECVAM in support of validation studies, the allocation of tasks to members and the financing of network activities.

Application for membership of EU-NETVAL is currently closed. A new call for members is anticipated during 2014/2015.

For further information on EU-NETVAL please contact us at

JRC-ECVAM-NETVAL@ec.europa.eu



http://ihcp.jrc.ec.europa.eu/our_labs/eurl-ecvam/eu-netval



JRC SCIENTIFIC AND POLICY REPORTS

EURL ECVAM Strategy for Replacement of Animal Testing for Skin Sensitisation Hazard Identification and Classification

Silvia Casati, Maurice Whelan
2013



<http://publications.jrc.ec.europa.eu/>

Joint Research Centre

In preparation

- Acute Toxicity
- (Dev)Neurotoxicity
- Biokinetics
- Fish Toxicity
-

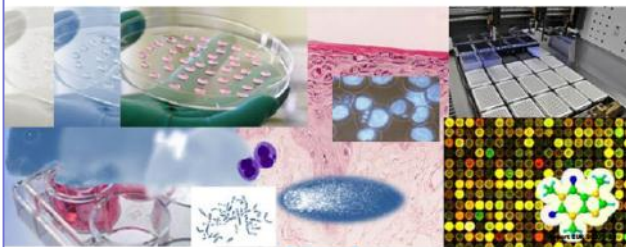


European Commission

JRC SCIENTIFIC AND POLICY REPORTS

EURL ECVAM Strategy to Avoid and Reduce Animal Use in Genotoxicity Testing

Raffaella Corvi, Federica Medda, Andrew Worth and Maurice Whelan
2013

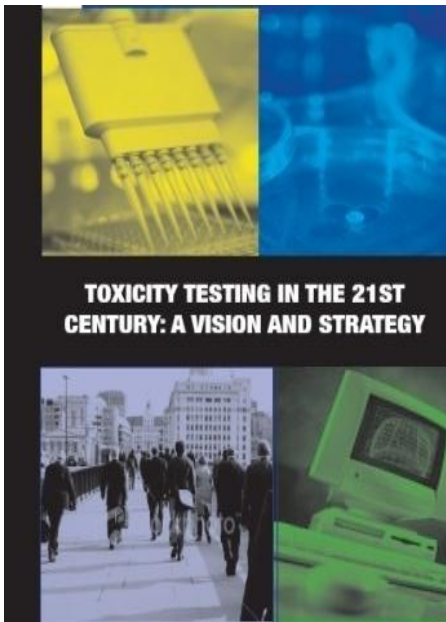


Joint Research Centre

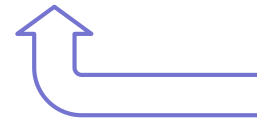
- Analysis of regulatory information needs
- Cross-sectorial scope
- Broad strategic aims
- Ambitious but pragmatic
- Solutions intended for regulatory acceptance
- Look towards international TGs and Guidance (IATA - Integrated Approaches to Testing and Assessment)

*Explicit
Transparent
Inclusive*

Toxicity Pathways (NRC 2007)



WHO/IPCS
Mode-of-Action
Framework
(Meek et.al. 2013)



Adverse Outcome
Pathways (Ankley
et. al. 2010)

Sufficient
Exposure
Triggers



Mechanistic Relevance - *Adverse Outcome Pathways*

- Being explicit about toxicological ***mode of action***



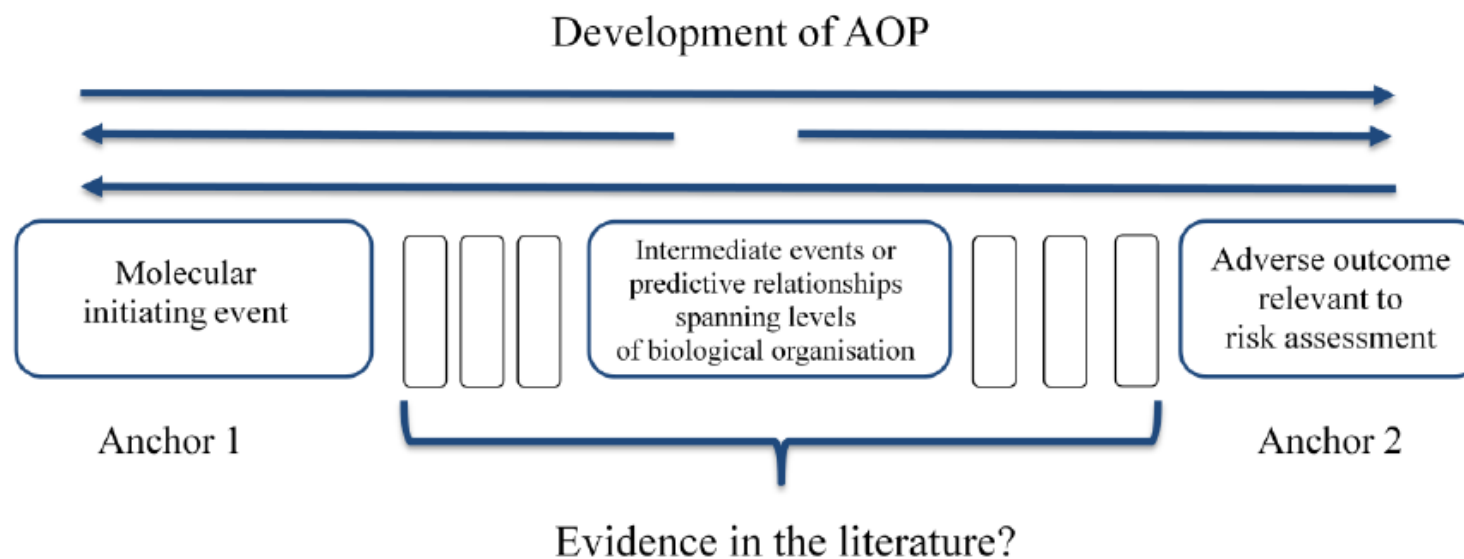
- to rationally design ***integrated prediction systems***
- fit for the purpose of ***supporting safety decisions***

... facilitating a shift towards a knowledge-driven paradigm for chemical risk assessment

Adverse Outcome Pathway Development Programme

Extended Advisory Group on Molecular Screening and Toxicogenomics

Co-chairs: Robert Kavlock (US EPA) & Maurice Whelan (EC JRC)





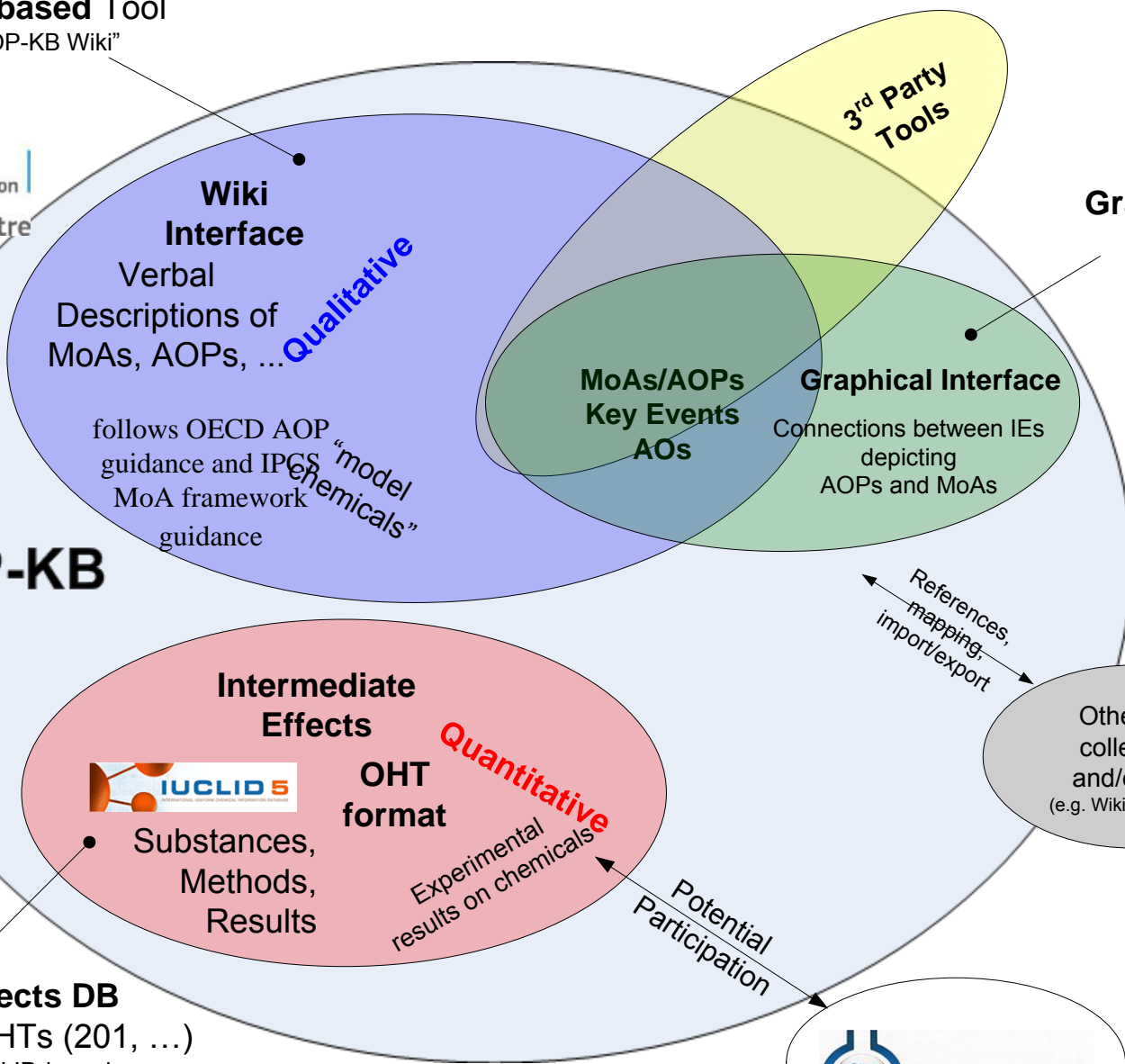
AOP Knowledge Base

Wiki-based Tool
"AOP-KB Wiki"



AOP-KB

Effects DB
using OHTs (201, ...)
IUCLID-based



3rd Party Tools

Graphical Tool(s)
Effectopedia,
ERDC tool, ...

Wiki Interface
Verbal
Descriptions of
MoAs, AOPs, ...
Qualitative
follows OECD AOP
guidance and IPCS
MoA framework
guidance
"model
chemicals"

**MoAs/AOPs
Key Events
AOs**

Graphical Interface
Connections between IEs
depicting
AOPs and MoAs

References,
mapping,
import/export

Other data
collections
and/or tools
(e.g. WikiPathways...)

**Intermediate
Effects**



**OHT
format**

Substances,
Methods,
Results

Quantitative
Experimental
results on chemicals

Potential
Participation



OECD

Extended Advisory Group on Molecular Screening and Toxicogenomics

Guidance for describing non-guideline in vitro test methods to facilitate their consideration in regulatory applications

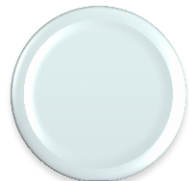
Introduction to Guidance

1. General information
 2. Test Method Definition
 3. Data interpretation and prediction model
 4. Test Method Performance
 5. Potential Regulatory applications
 6. Bibliography
 7. Supporting information
- Glossary of terms

Work in progress!

Talk Outline

Validation of new tools ...



Overview of EURL ECVAM



Validation in a regulatory context



Validation in a research context

Safety Assessment Ultimately Replacing Animal Testing



EUROPEAN COMMISSION
 Research & Innovation
 SEURAT-1
 Paracelsus (1493-1541):
 "The *dose* makes the poison"

EUROPEAN COMMISSION
 Research & Innovation
 Cosmetics Europe
 SEURAT-1
 Paracelsus (1493-1541):
 "The *dose* makes the poison"

Towards the Replacement of *in vivo* Dose Systemic Toxicity Testing

Towards the Replacement of *in vivo* Repeated Dose Systemic Toxicity Testing

Toxicology in the 21st century: Mechanism-driven Toxicology defines the safe dose
Volume 1 2011

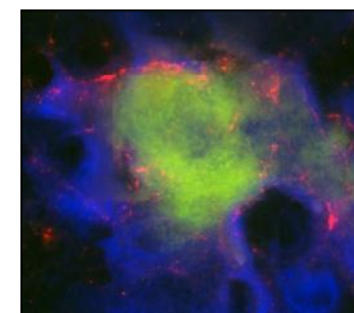
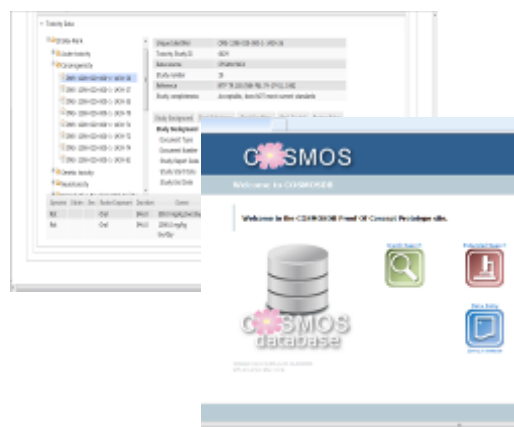
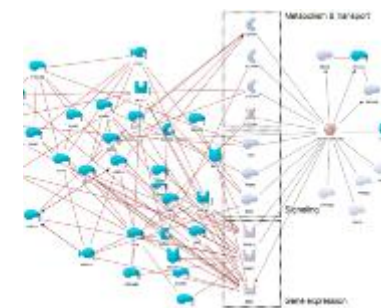
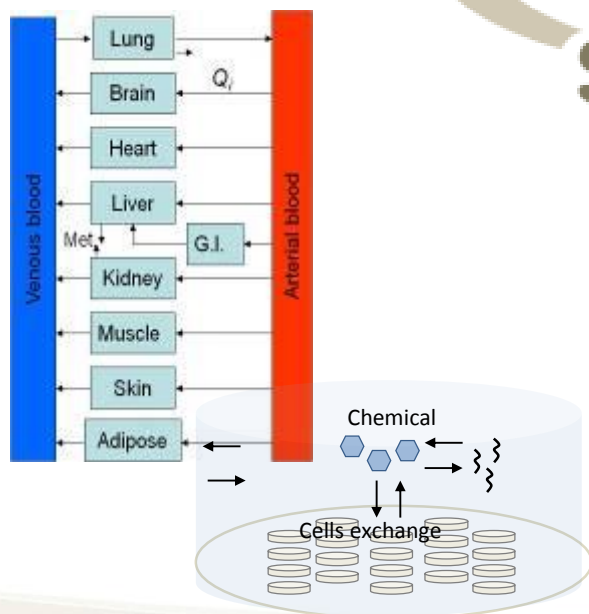
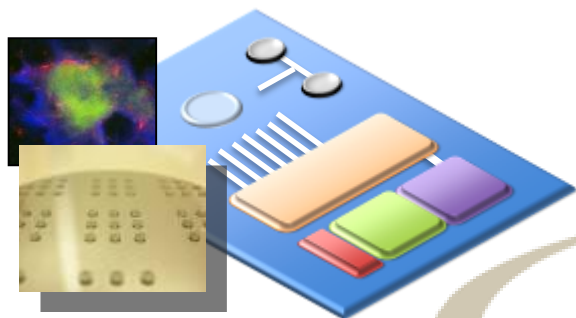
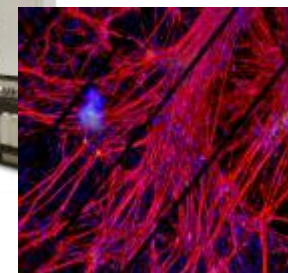
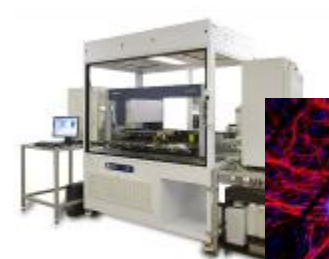
Toxicology in the 21st century: Mechanism-driven Toxicology defines the safe dose
Volume 2 2012

Toxicology in the 21st century: Mechanism-driven Toxicology defines the safe dose
Volume 3 2013

Health Programme: Advanced therapies and systems medicine (H4)
 Coordinator: Action COSMIC Grant Agreement N° 267044
 DG Research & Innovation

Health Programme: Biocentric therapies and systems medicine (H4)
 Coordinator: Action COSMIC Grant Agreement N° 267044
 DG Research & Innovation

Scientific Tools



... how to convert them into solutions for safety assessment?

SEURAT - The Strategy

*The SEURAT strategy is to adopt a toxicological **mode-of-action framework** to describe how any substance may adversely affect human health, and to use this knowledge to develop complementary theoretical, computational and experimental (in vitro) models that predict quantitative points of departure needed for safety assessment.*

Validation - *Proving Concepts*



- **Level 1: Knowledge**

Theoretical MoA descriptions, which might be further refined or confirmed based on the outcome from SEURAT-1 activities

- **Level 2: Prediction**

Integrated assessment and testing systems for mechanistic grouping of chemicals and for quantitative prediction of adverse effects.

- **Level 3: Application**

Application of the information derived from methods and predictive systems to support safety assessment processes and decisions.

Predictive toxicology – Case studies

Prediction Goal	Please clearly indicate the purpose of the system/strategy/method being developed with respect to the toxicological effect(s) it is designed to predict.
Mechanistic basis	Please describe the mechanistic basis underpinning the approach, mentioning for example any relevant mode(s) of action that are being considered.
Scientific approach	Please briefly describe the scientific approach to be taken including, for example, the design rationale, what methods will be employed, the basis for predicting an outcome and interpretation of data, and, if foreseen, any anticipated validation activity to assess predictive performance.
Chemical selection	Please identify what chemicals you intend to use for development and assessment of the system, including, for example, positive and negative reference chemicals.

9 case studies defined – deliver Q1 2015 !

Prediction Goal - designing for purpose

- Profile the bioactivity of a chemical to associate it with an activity category that is related to a toxicity endpoint.
- Identify a chemical as being associated, or not, with a MoA that is likely to cause an adverse health outcome.
- Rank chemicals within a bioactivity/MoA category with respect to their potency/likelihood to cause toxicity.
- Predict a quantitative *in vivo* point-of-departure for a chemical with respect to a specific toxicity endpoint.

Case studies on *Application Level*

∴ **DATA RICH**

Make use of information from the SEURAT-1 *in vitro* molecular screening and 'omics data, as well as computational models to strengthen the weight of evidence approach and to improve the robustness of a read-across or chemical categorisation and validating the case.

∴ **DATA POOR**

An ab initio assessment using the Integrated Assessment Strategy developed by SEURAT-1 that would highlight gaps for future development and illustrate overall progress made in SEURAT-1.

Predictive but Pragmatic



In summary ...

Validation is a fundamental part of the scientific process
.... but be scientific, smart, and purpose driven

Innovation is indeed needed,
as an evolution not revolution,
to avoid



<http://vickybeeching.com/blog/wp-content/uploads/2012/02/baby-out-with-bath-water.jpeg>

Thank you !

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