



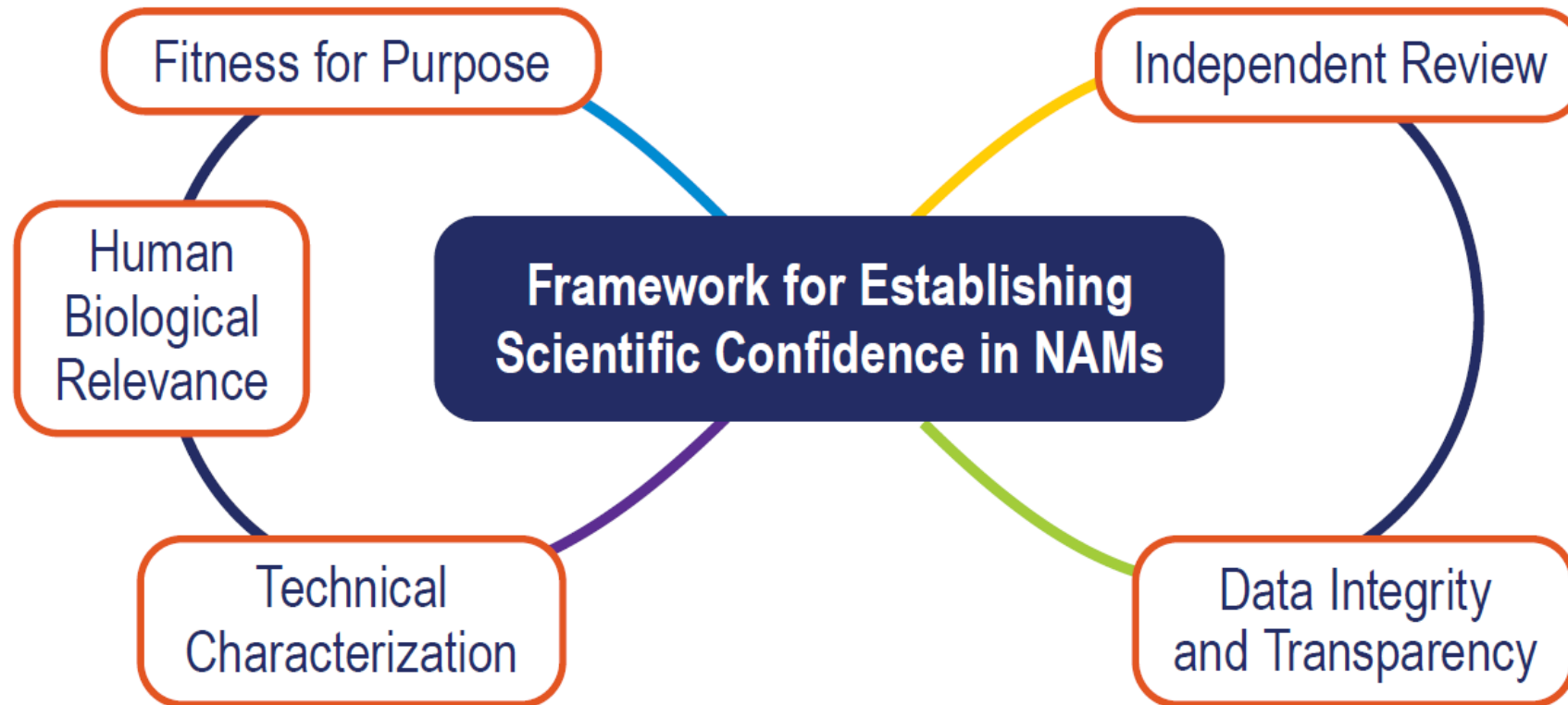
Establishing scientific confidence in New Approach Methodologies (NAMs)

Transparency, data integrity, external review and ring trials

João Barroso (Joint Research Centre, EURL ECVAM)

SACATM meeting, 22 September 2022

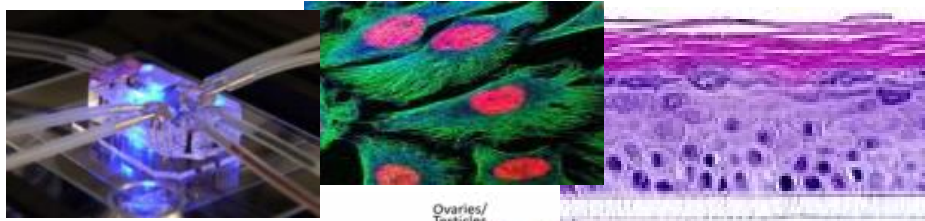
A framework for establishing scientific confidence in new approach methodologies



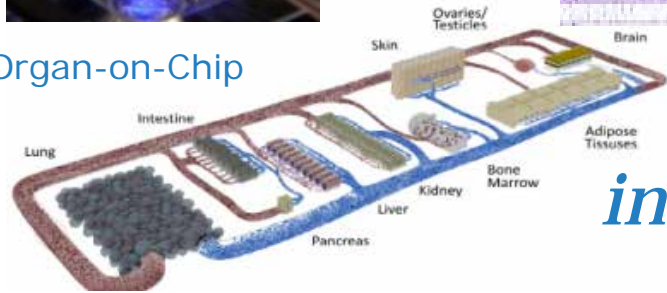
van der Zalm AJ, Barroso J, Browne P, Casey W, Gordon J, Henry TR, Kleinstreuer NC, Lowit AB, Perron M, Clippinger AJ. Arch Toxicol. 2022. doi: 10.1007/s00204-022-03365-4

<https://link.springer.com/content/pdf/10.1007/s00204-022-03365-4.pdf>

The modern safety assessment toolbox

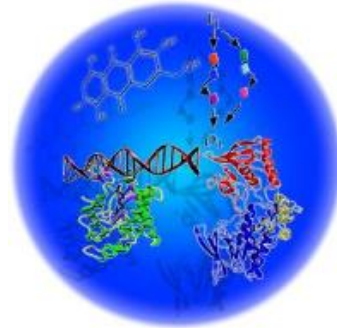


Organ-on-Chip



in vitro

in silico

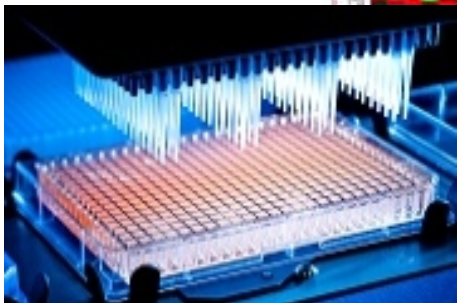
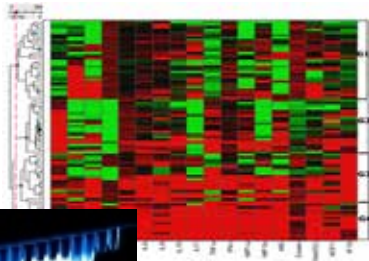


clinical data

existing animal data



'omics

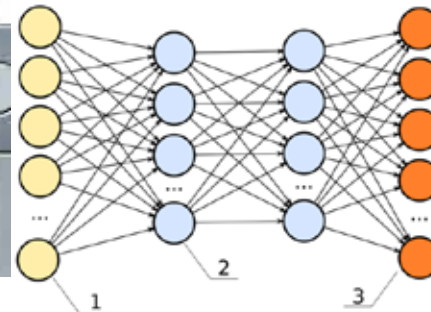


3 High Throughput Screening

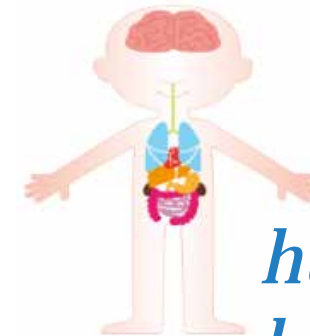


integration

machine learning/AI



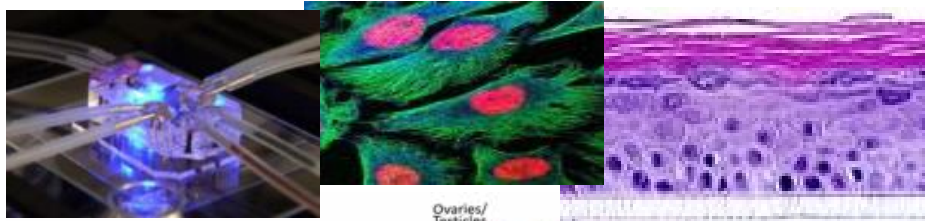
biokinetics



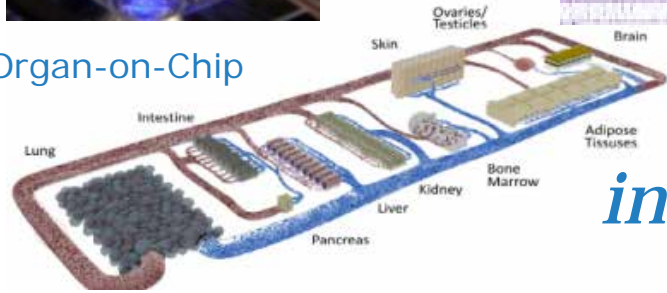
human biomonitoring



The modern safety assessment toolbox

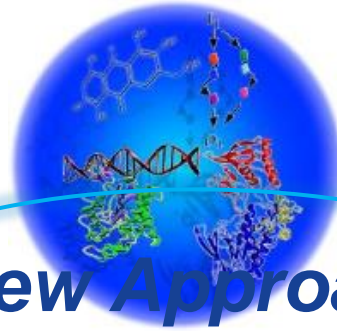


Organ-on-Chip



in vitro

in silico



New Approach Methodologies (NAMs)

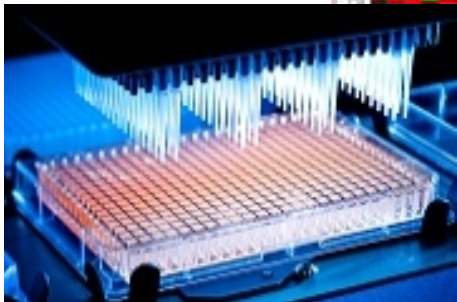
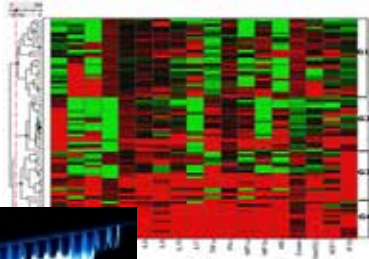


clinical data

existing animal data



'omics

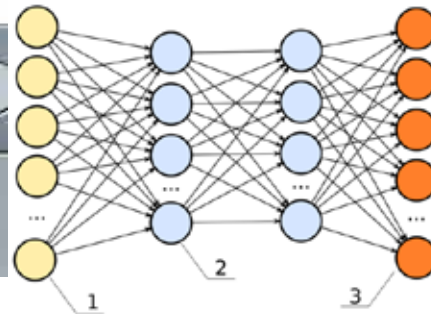


4 High Throughput Screening

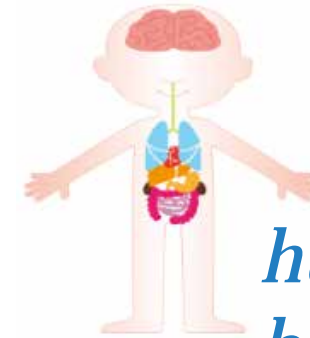
machine learning/AI



integration



biokinetics

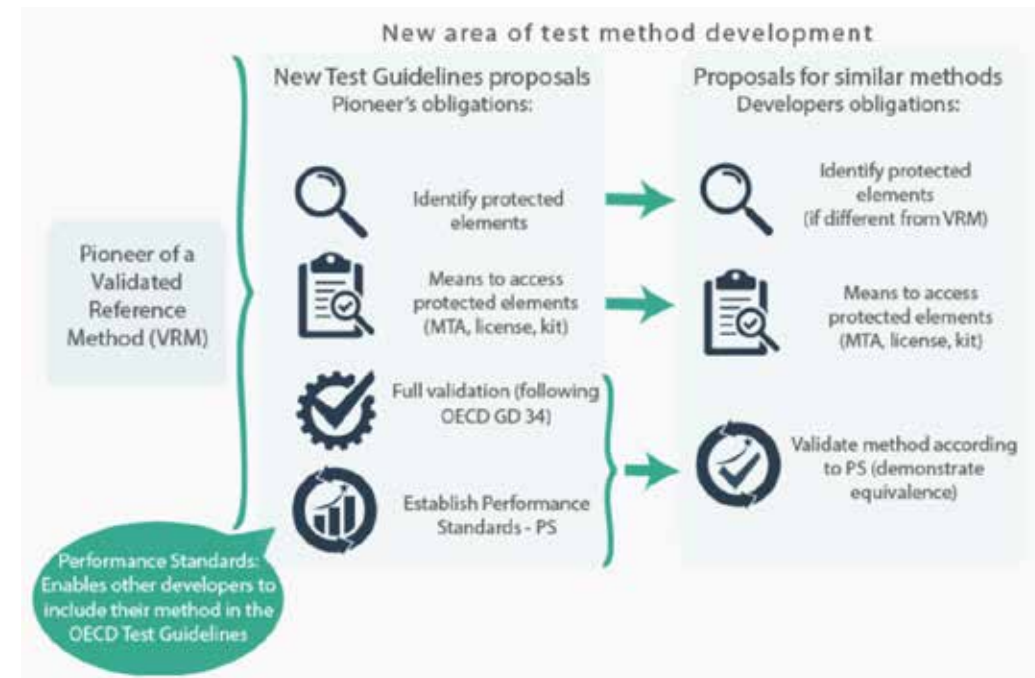


human biomonitoring



Transparency: OECD policy for confidential information in candidate Test Guidelines

- Method developers encouraged to use other means than confidentiality to protect their intellectual property
- OECD will host confidential information on a protected webpage accessible to National Coordinators only during Test Guideline development
- Once the Test Guideline is adopted, this information will be made publicly available



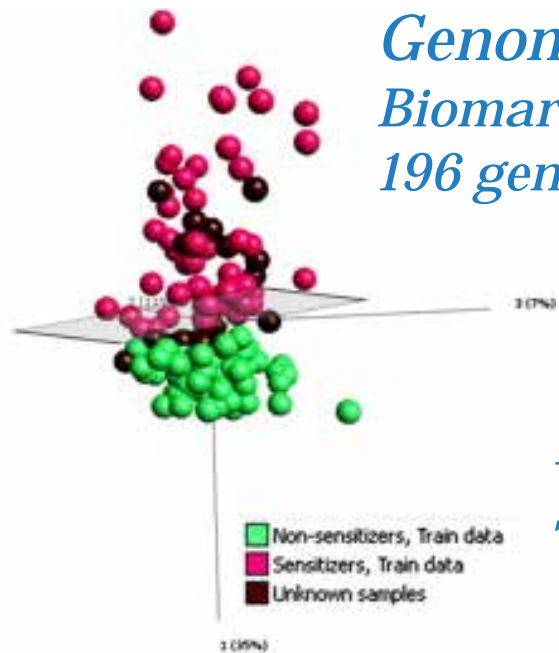
Source: <https://www.oecd.org/chemicalsafety/testing/intellectual-property-in-oecd-test-guidelines.htm>

Independent scientific review

- Important part of confidence building process
- Appropriate level of external review depends on the method and context of use
- Might include publication in peer-reviewed journal or review by an independent scientific advisory panel
- International adoption by OECD typically needs formal peer review
- Method developers may fund but should not manage peer review

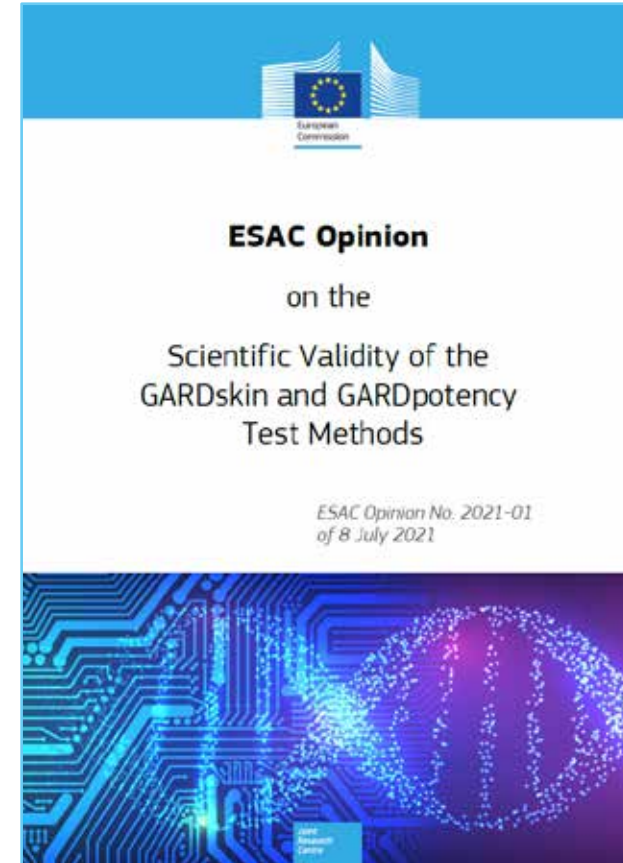
ESAC review of the GARD

Genomic Allergen Rapid Detection (GARDskin)



*Genomic based
Biomarker signature
196 genes*

*Classification based on
Machine Learning algorithm
Support Vector Machine*

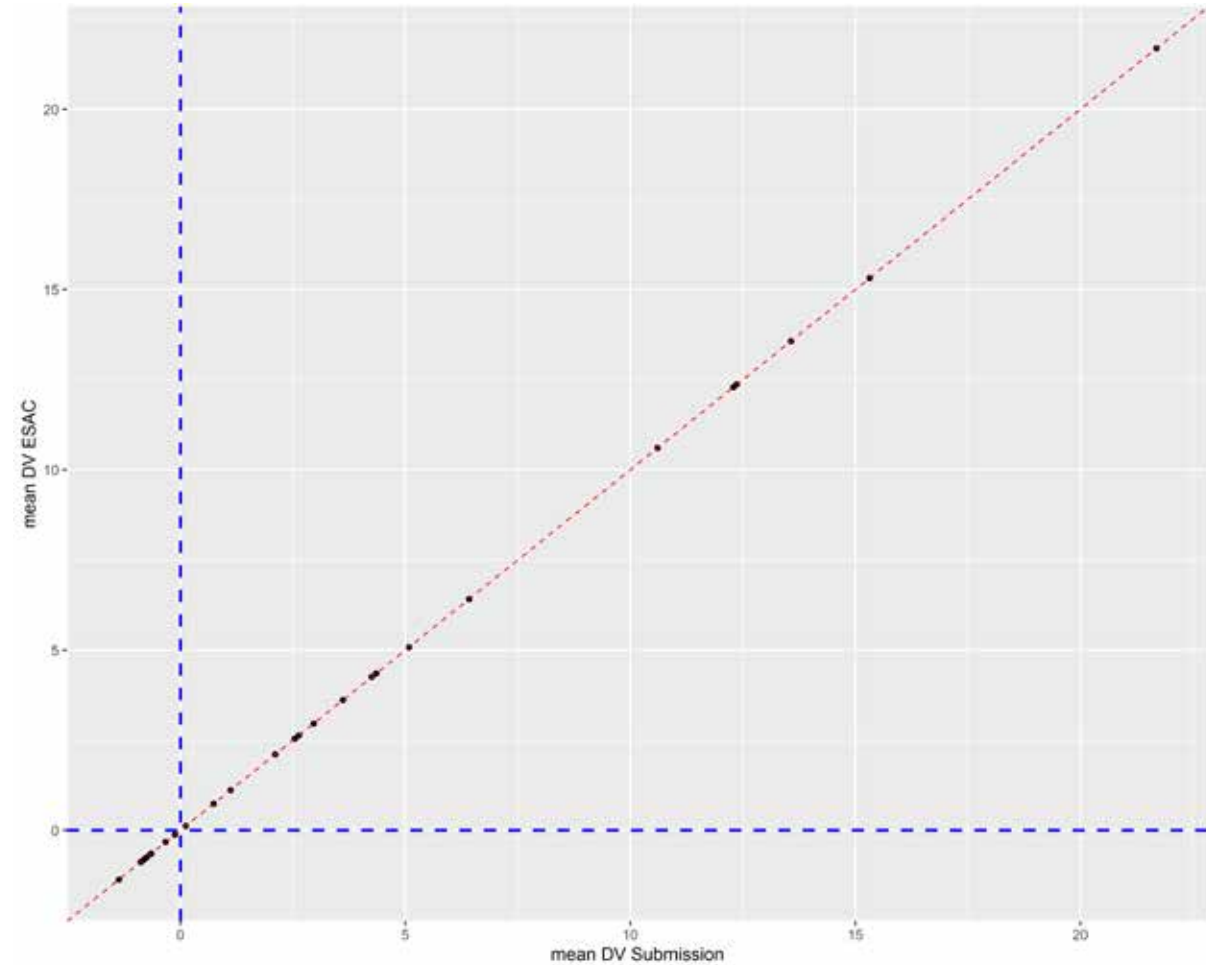


<https://ec.europa.eu/jrc/en/science-update/machine-learning-and-omics-arrive-field-regulatory-toxicology>

Transparency

Independently reproduce data

- External implementation and training of the models
- Processing of the raw data
- Replicate predictions obtained in the validation study



TSAR - Tracking System for Alternative methods towards Regulatory acceptance

Communicate transparently and, as far as possible, make information publicly available

The screenshot shows the TSAR website interface. At the top, there is the European Commission logo and a search bar. Below this is a blue header with the text "TSAR - Tracking System for Alternative methods towards Regulatory acceptance". The main content area is titled "Genomic Allergen Rapid Detection test".

Topic: Sensitisation

Test Method Number: TM2011-09 (EU)
Short Name of TM: GARD
Year received: 2011
Responsible Organisation: [EURL ECVM - European Union](#)
Protocol(s)/SOP(s): [GARDskin Assay Protocol](#)
General Comments: Please note that the GARDskin Assay Protocol available in the link above is a revised version provided by the test method developer after completion of the ESAC peer review to address comments made by the ESAC.

Method Description

The Genomic Allergen Rapid Detection (GARD) is a transcriptomics-based *in vitro* assay proposed to assess the skin sensitisation potential/potency of chemicals. GARD addresses the third key event of the skin sensitisation Adverse Outcome Pathway (activation of dendritic cells), step 5 (biochemical pathways related to skin sensitisation) and step 6 (immune recognition of chemical allergens and maturation of dendritic cells (DCs)).

The test method has two elements: the so-called GARDskin to assess skin sensitisation potential (first submission in October 2011) and GARDpotency to assess skin sensitisation potency (first submission in July 2018).

The test method is based on the nCounter system and measures the expression level of a panel of genes in the human myeloid cell line MUTZ-3 exposed to chemicals. In GARDskin, the expression of a panel of 200 genes (the GARD Prediction Signature, GPS) is used as input to a prediction model based...

[Read more]

Track Approval Status

The progress bar shows five steps: 1. SUBMISSION (Finalised) with a green checkmark; 2. VALIDATION (Finalised) with a green checkmark; 3. PEER REVIEW (Finalised) with a green checkmark; 4. RECOMMENDATION (Ongoing) with a grey circle and arrow; 5. REGULATORY ACCEPTANCE/STANDARDS (Ongoing) with a blue circle and arrow.

Step	Expand All
Submission	+
Validation	+
Peer-review	+
Recommendation	+
Regulatory acceptance/Standards	+

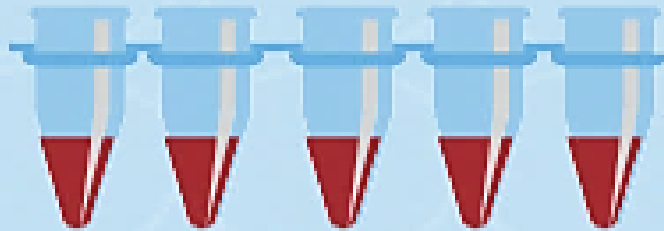
Data integrity in validation studies

- According to OECD GD 34, validation studies should follow the principles of GLP
- Mostly not done in the past but not a problem because studies were coordinated by independent parties
- Now managed by commercial parties
à trustworthiness becomes questionable
- Important to demonstrate the integrity and credibility of the results, from the raw data through to the final report

Standards to ensure **Reliability** and **Data Integrity**



GIVIMP



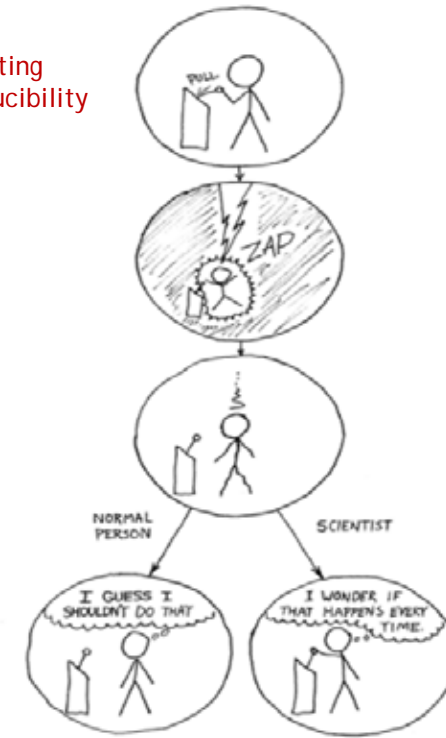
**OECD Guidance Document
on Good In Vitro Method Practices**

The OECD has published guidance on Good In Vitro Method Practices (GIVIMP) for the development and implementation of in vitro methods for regulatory use in human safety assessment

Reproducibility and ring trials

- Demonstrating reproducibility is essential
- Ring trials are the most time-consuming and expensive part of a validation study and are often more a reflection of laboratory quality or expertise than of a NAM's reproducibility
- Assessing BLR is not always possible (e.g. for certain types of technology) and not necessary with automation
- Properly designed training and transfer studies are essential and informative
- Better characterisation of method reproducibility and critical steps by developer, e.g. sensitivity analysis of all parameters that can affect result
- Proficiency testing adds confidence on capacity of a laboratory to perform test

Testing
reproducibility



Thyroid Validation Study, a collaborative effort!

14 method developers

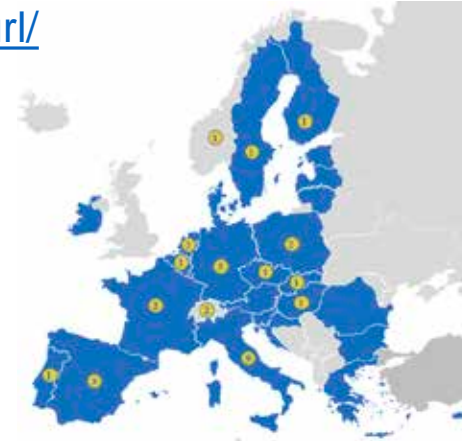



scientific experts

1. University of Pisa, Pisa, Italy.
2. Karolinska Institutet, Solna, Sweden.
3. OECD, Paris, France.
4. National Museum of Natural History, Paris, France.
5. Bayer AG, Wuppertal, Germany.
6. US Environmental Protection Agency, Durham, USA.
7. Masaryk University, Brno, Czech Republic.
8. University of Antwerp, Antwerp, Belgium.
9. Charité-Universitätsmedizin, Berlin, Germany.
10. University of Catania, Catania, Italy.
11. Syngenta, Cambridge, United Kingdom.
12. German Federal Institute for Risk Assessment, Berlin, Germany.
13. Health Canada, Ottawa, Canada

15 EU-NETVAL labs

<https://ec.europa.eu/jrc/en/eurl/ecvam/alternative-methods-toxicity-testing/eu-netval>



PART 1: Method Definition & Reliability

- ü Produce SOPs
- ü Method optimization if needed
- ü Aim: assessing robustness and reliability
- ü Experimental study of 5 valid runs

PART 2: Relevance

- ü Aim: assessing overall relevance
- ü Testing of 30 blind-coded chemicals
- ü Experimental study of 3 valid runs

18 Methods

TRACKING SYSTEM FOR ALTERNATIVE METHODS TOWARDS REGULATORY ACCEPTANCE

TSAR tracks the progress of alternative, non-animal methods, for testing chemicals or biological agents such as vaccines towards acceptance as a recognised test method for use in various sectors



Final thoughts

- Validation as a process of building scientific confidence is essential to facilitate acceptance and ensure sound science-based decisions
- Validation needs to keep pace with rapid scientific progress, e.g. emergence of Defined Approaches (data integration), computational models, new technologies such as Organ-on-Chip
- Important to maintain scientific integrity, credibility and usefulness while making process more efficient
- Frame validation as a process to characterize and reduce uncertainty rather than a ring trial to demonstrate "toxicological equivalence"
- Validation \neq regulatory acceptance and use

Thank you



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