Red Española para el Desarrollo de Métodos Alternativos a la experimentación animal

Los métodos alternativos a la experimentación animal ante las nuevas normativas internacionales

IV Jornada de REMA, X Aniversario

Colegio Oficial de Veterinarios de Madrid

1 de diciembre de 2009

Documentos disponibles en http://www.remanet.net/

Development and Validation of Alternative Methods

10th Anniversary REMA, Madrid, 1 December 2009

Sharon Munn

Alternative Methods and ECVAM
The Institute for Health and Consumer Protection (IHCP)

Science for a healthier life

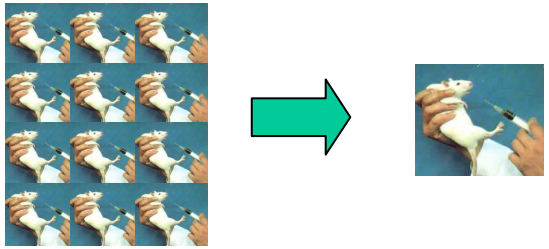
OUTLINE

- History
- Validation principles
- Successes and next challenges
- ECVAM Role

Two British scientists, Bill Russell and Rex Burch introduced the "3Rs" as a framework for considering the humane use of animals.

Refine

Reduce



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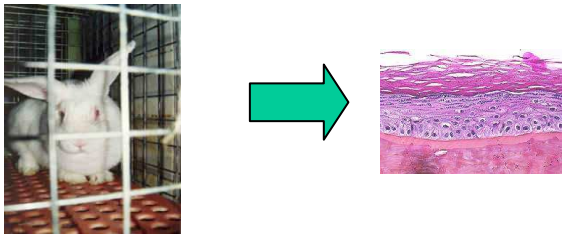


86/609/EEC

Russell, W. M. S. and Burch, R. L. 1959. The principles of humane experimental technique Special Edition, Universities Federation for Animal Welfare, Potters Bar, England

ECVAM

- Founded in 1991 to promote 3R methods primarily by confirming their scientific validity
- From 1991 to 2009 ECVAM was hosted within one JRC scientific Unit of the former Environment Institute (1991-1998) and of the Institute for Health and Consumer Protection (1998-2008)
- Since 2009 ECVAM is a Centre hosted by the Institute for Health and Consumer Protection and is served by mainly two scientific Units
- ECVAM has its own scientific advisory committee (ESAC)

ECVAM

- So far developed/optimised and/or validated 34 methods alternative to animal testing according to generally accepted validation principles
- Most methods have similar toxicological endpoints, i.e. skin and eye irritation; many methods are not replacement methods
- Maintains a database on alternative test methods (DB-ALM) and tracking system (TSAR)
- Promotes method development through own research as well as through participation in RTD projects that will yield new methods
- Contributes to the regulatory acceptance of alternative methods

ECVAM's MISSION STATEMENT

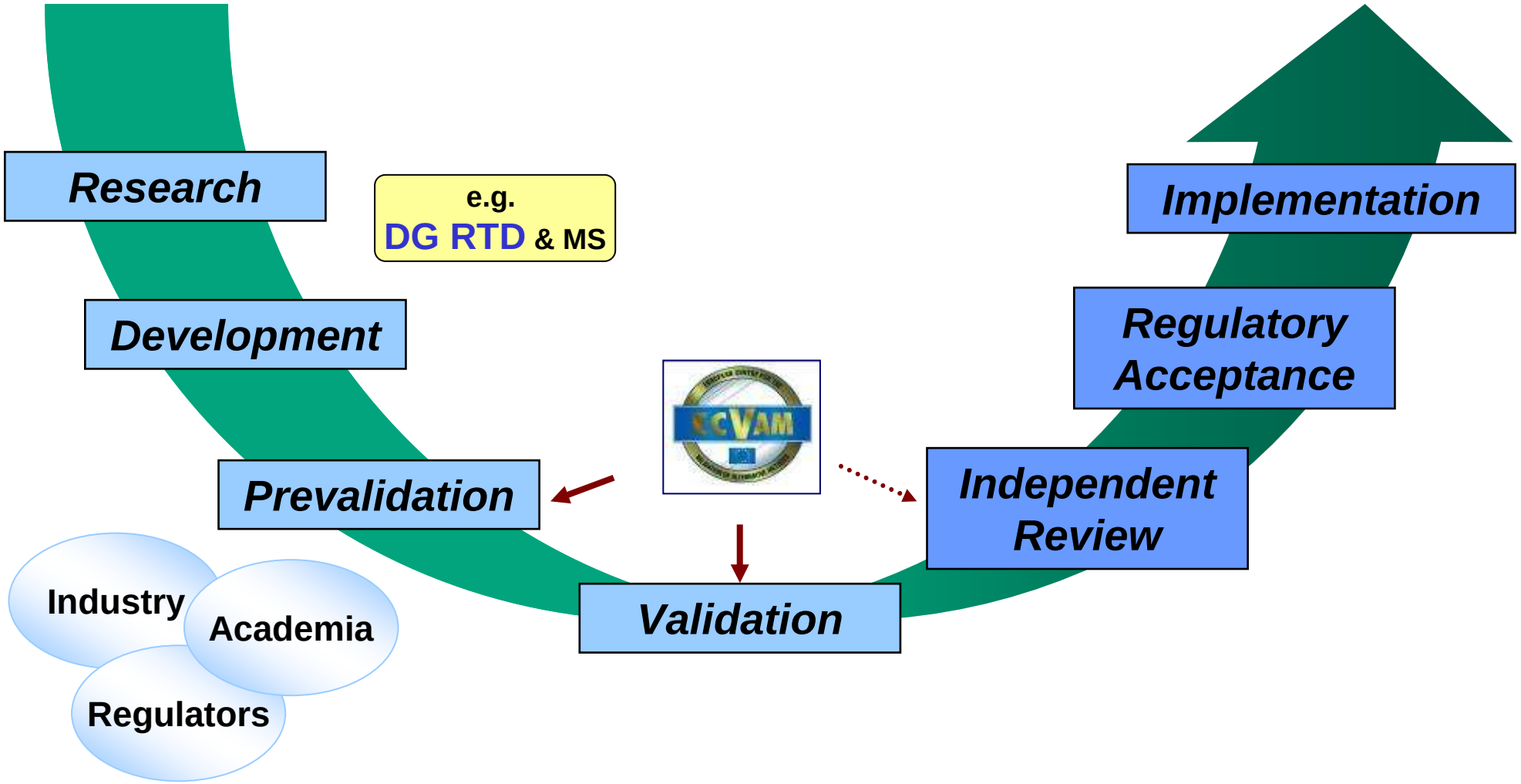
To support the EU policies in the field of Consumer protection, Environmental protection and Animal protection

by validating alternative methods for safety testing that implement the 3Rs and provide the same or a better basis for risk assessment and risk management as current methods

and by promoting their development, their application in industry and their acceptance by regulators.

OUTLINE

- History
- Validation principles
- Successes and next challenges
- ECVAM Role


Research
e.g.
DG RTD & MS
Development
Prevalidation
Industry
Academia
Regulators
Validation
Independent Review
Regulatory Acceptance
Implementation

Information requirements that validation studies endeavour to satisfy

Test method definition	*Module 1 – Test definition:* ***test system, SOP, prediction model, development, possible use, limitations, etc.***
Reliability *Concordance/ Discordance*	*Module 2 – Within laboratory reproducibility:* ***sufficiently standardized to give reproducible results in one lab ?***
	Module 3 – Transferability: ***transferable, and yes, how readily?***
	Module 4 – Between laboratory reproducibility: ***how reproducible between labs?***
(Predictive) Relevance *Accuracy*	*Module 5 – Predictive capacity:* ***Specificity, Sensitivity, Overall Accuracy***
	Module 6 – Applicability domain/Limitations: ***Which xenobiotics can NOT be tested?***
Performance criteria	*Module 7 – Performance Standards:* ***Performance Acceptance Criteria for new tests that are sufficiently similar to the validated one***

OUTLINE

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ECVAM activities & involvement, Per endpoint, 2009

	Development	Prevalidation	Validation	Reg. acceptance
Skin Corrosion	✓	✓	✓	✓
Acute Phototoxicity	✓	✓	✓	✓
Skin Absorption / Penetration	✓	✓	✓	✓
Skin Irritation	✓	✓	✓	✓
Eye Irritation	✓	✓	✓*	✓*
Acute Toxicity	✓	✓	✓*	
Genotoxicity / Mutagenicity	✓	✓	✓*	✓*
Skin Sensitisation	✓	✓	✓*	✓*
Reproductive & Developmental	✓	✓	✓*	✓*
Toxicokinetics / Metabolism	✓	✓	✓*	
Carcinogenicity	✓	✓*	✓*	
Subacute & Subchronic Toxicity	✓			
Biologicals, vaccines	✓	✓	✓	✓

* Reduction / refinement alternatives

Human health effects

Skin Corrosion

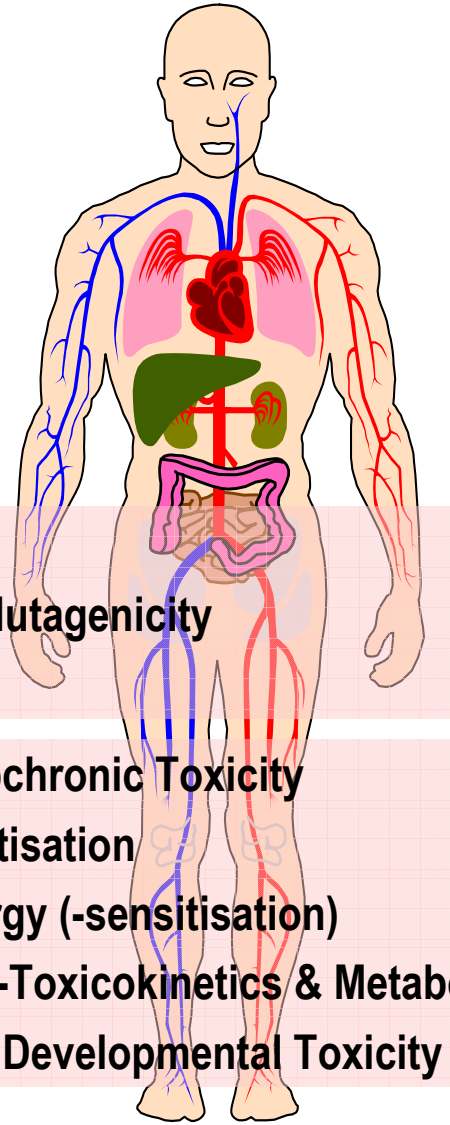
Acute Phototoxicity

Skin Absorption / Penetration

Skin Irritation

Photogenotoxicity

Eye Irritation



Acute Toxicity

Genotoxicity / Mutagenicity

Subacute & Subchronic Toxicity

Skin Sensitisation

Photo-allergy (-sensitisation)

Carcinogenicity-Toxicokinetics & Metabolism

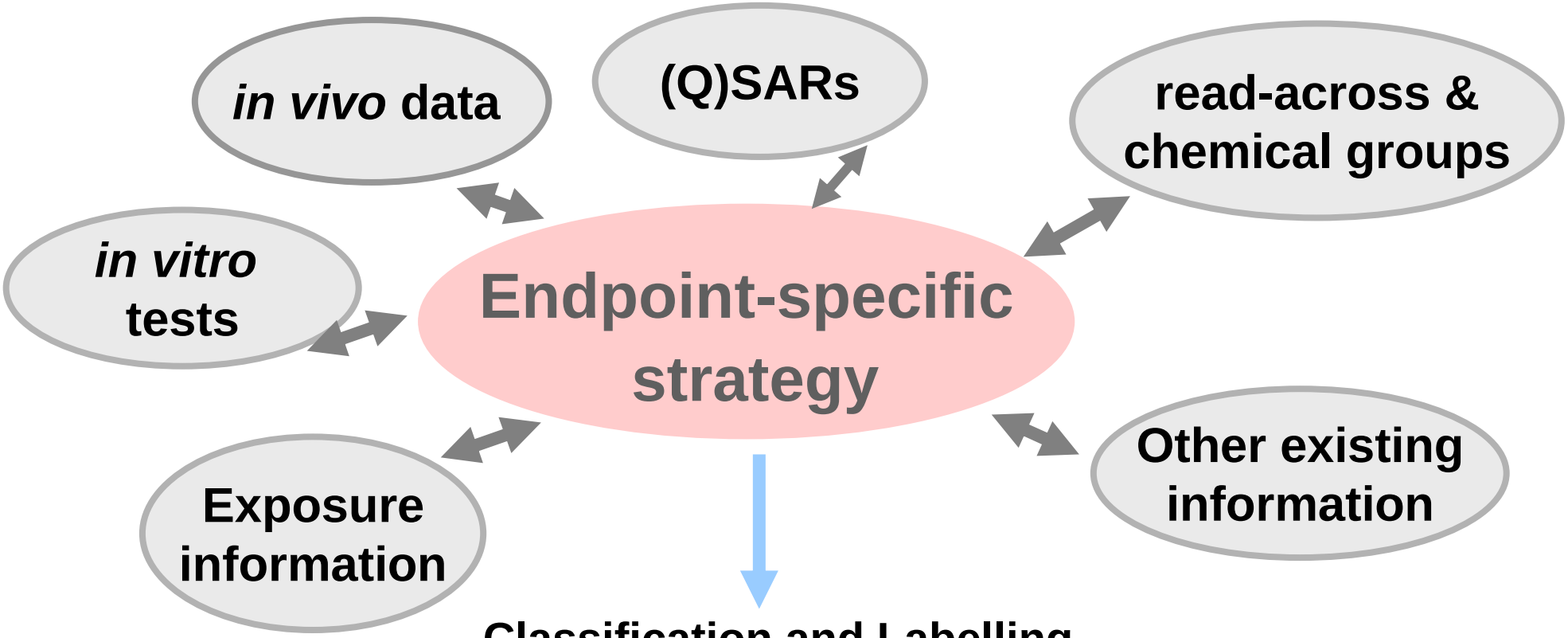
Reproductive & Developmental Toxicity

Biggest challenge

Systemic Toxicity - Systems Biology

- Metabolism
- Multiple modes of action
- Dose/response
- One to one replacement not possible
- Battery of test methods
- Tiered testing strategies
- Combination of disciplines (*in vitro/in silico/in vivo/PBPK models*)
- **Integrated testing strategies**

Integrated Testing Strategies

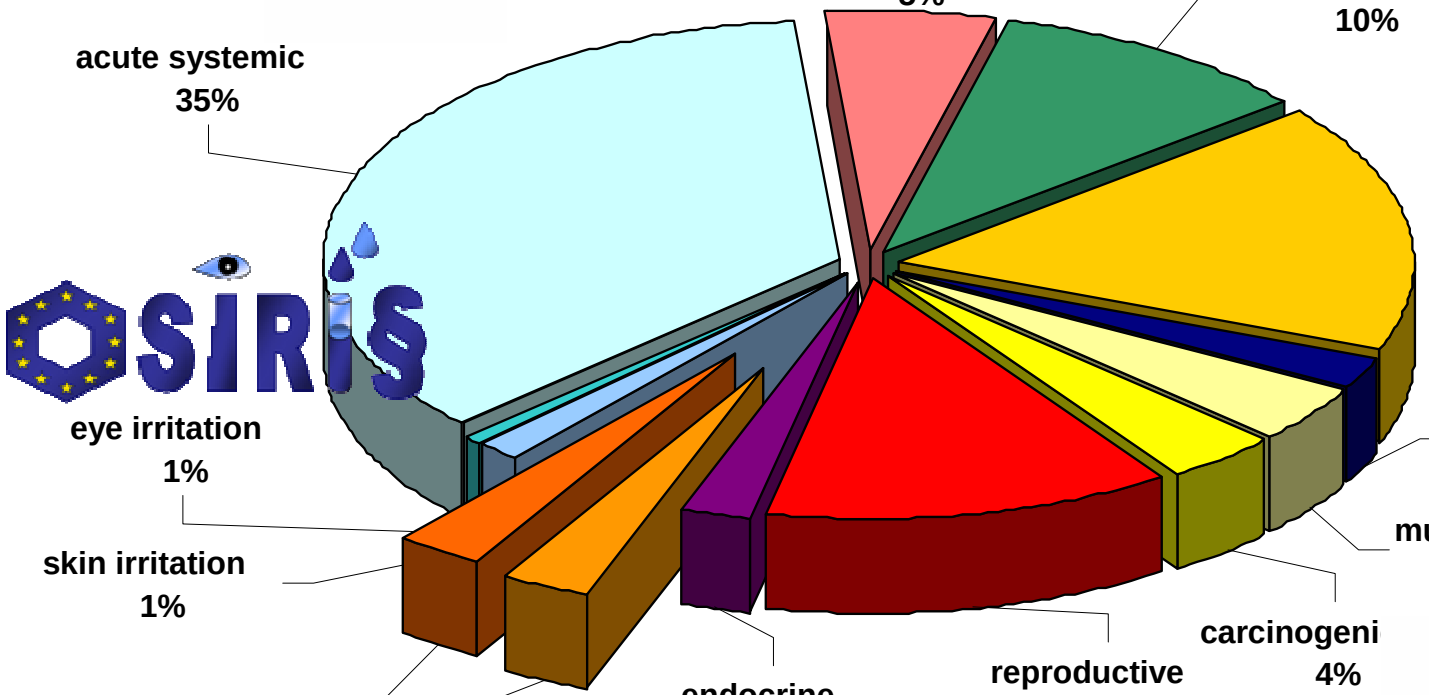


Classification and Labelling,
Risk Assessment of Chemicals and Persistent Bioaccumulative Toxic Chemicals

Risk Management Measures

New/emerging technologies

- Human cell-based metabolically competent liver cells
- Human stem cell-derived neurons/micro electrode arrays
- 3D in vitro tissue models
- Automation of in vitro methods (HTS/HCS)
- Development of computational methodology (in silico, QSAR)
- 'Omics', genomics, proteomics, metabonomics



SIXTH FRAMEWORK PROGRAMME

PULMO-NET

PREDICTOMICS

PREDICT-IV

Vitrocellomics

carcinoGENOMICS

RePrOTect

ESNATS

SEVENTH FRAMEWORK PROGRAMME

EC Call for proposals on Alternative Testing Strategies in field of repeated dose systemic toxicity– 7th Framework programme

- COLIPA matched funding to EC (Eur 25 + 25 million)
- Open call – 30 July 2009 to 3 Feb 2010

Validation of Integrated Testing Strategies?

- Validate ITS or Building blocks of ITS or both?
- Building blocks (reliability (modules 1-4) sufficient?)
- Predictive capacity – validate against what?
- Validation should be 'fit for purpose'
- Need case studies

Overcoming Barriers to Validation of Non-animal Partial replacement Methods/Integrated testing Strategies: The Report of an EPAA-ECVAM Workshop. ATLA, 37,437-444,2009

OUTLINE

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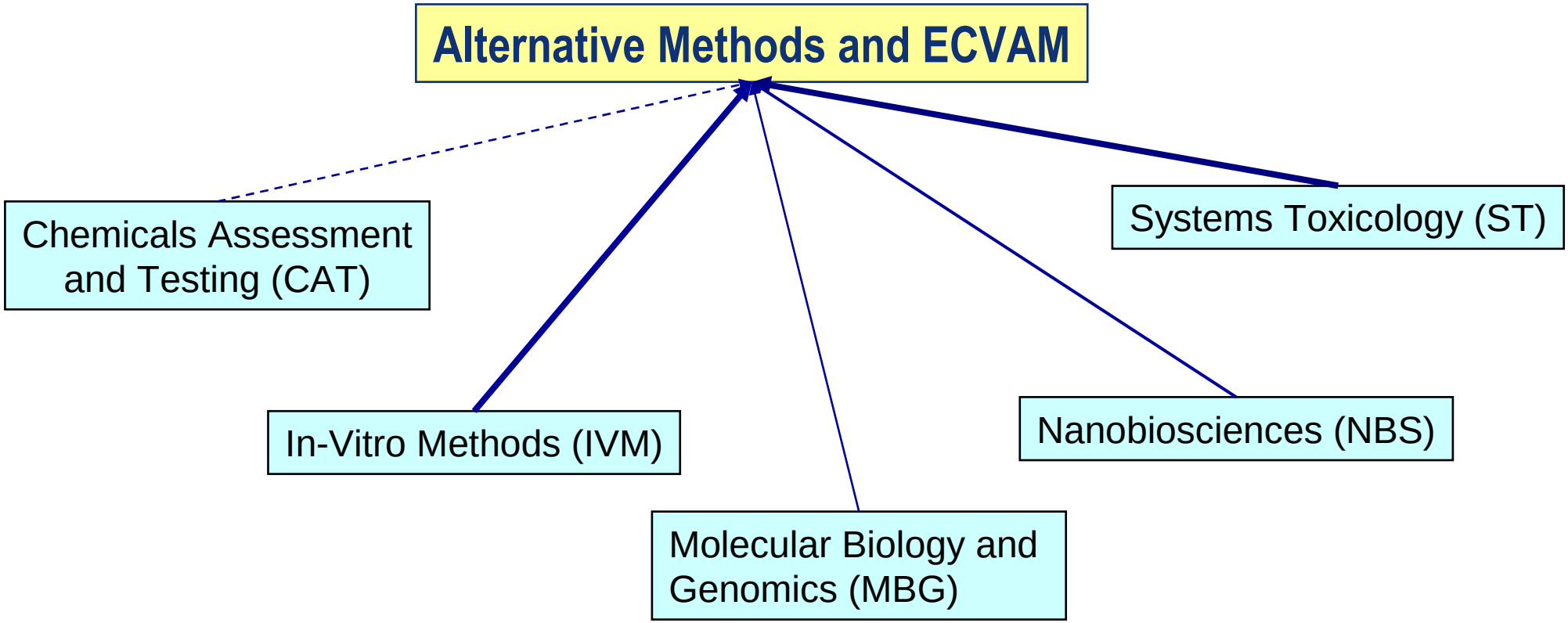
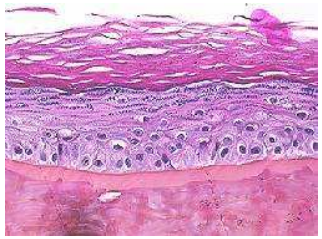
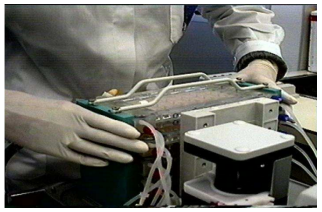
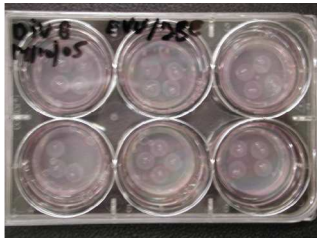
JRC-IHCP Units Supporting ECVAM

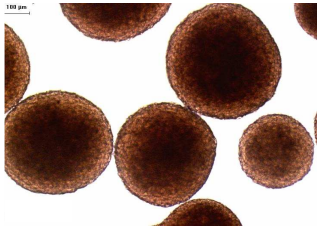


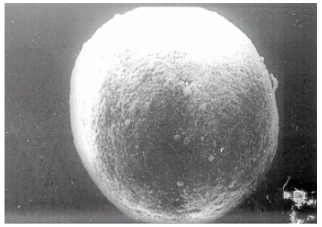


ECVAM has now full access to the broad range of competencies available across the Institute.







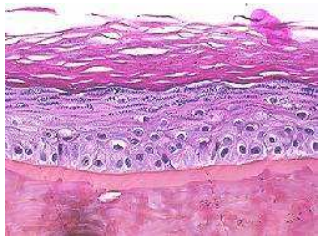


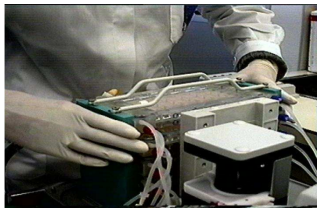
The relevant competencies include:

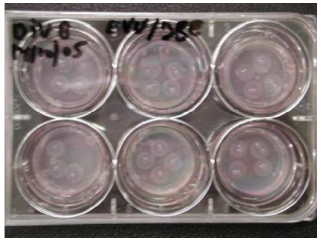
- **at cellular level: in vitro methods, working with many different (human) cell systems and their automation towards high throughput;**
- **at sub-cellular level: "omics" (metabonomics, genomics);**
- **at molecular or chemical level: computational chemistry including QSAR.**

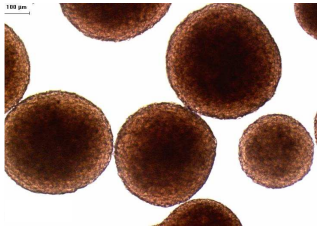
PBPK and modeling expertise to help connect the cellular and sub-cellular levels with the organ or organism level.

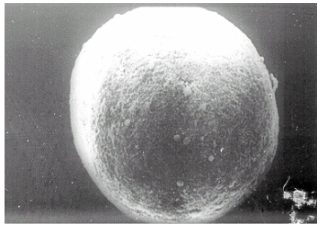
Wealth of experience on validation of alternative methods









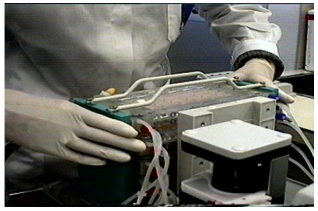


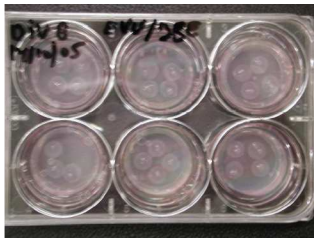
Validating alternative methods that provide the same or better basis for risk assessment as current methods

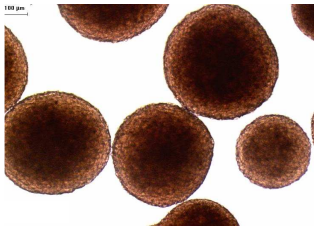
INNOVATION: contribute to methods/testing strategies that reduce reliance on in vivo animal studies even for complex endpoints

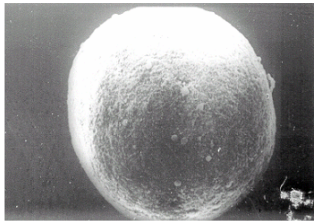
VALIDATION: continue to manage and coordinate scientific validation of submitted methods, assessing robustness, reliability, predictive capacity of methods and regulatory relevance, promoting regulatory acceptance

COMMUNICATION: engage with regulators/risk assessors, test developers, test users/risk assessors, promote dialogue/cross talk through workshops, promote uptake of methods though dissemination



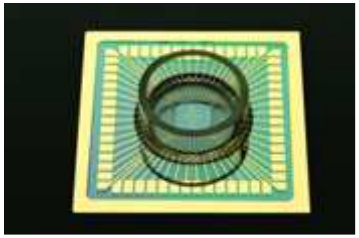






Concluding remarks

- **Integrated testing strategies required to address complex endpoints**
- **Emerging technologies give opportunity to make a gear change in progress**
- **A major challenge lies in the integration of the data and its interpretation in relation to specific regulatory questions**
- **Risk assessors need to engage in dialogue to give a steer to increase chance of relevant outcomes (both development and validation aspects)**

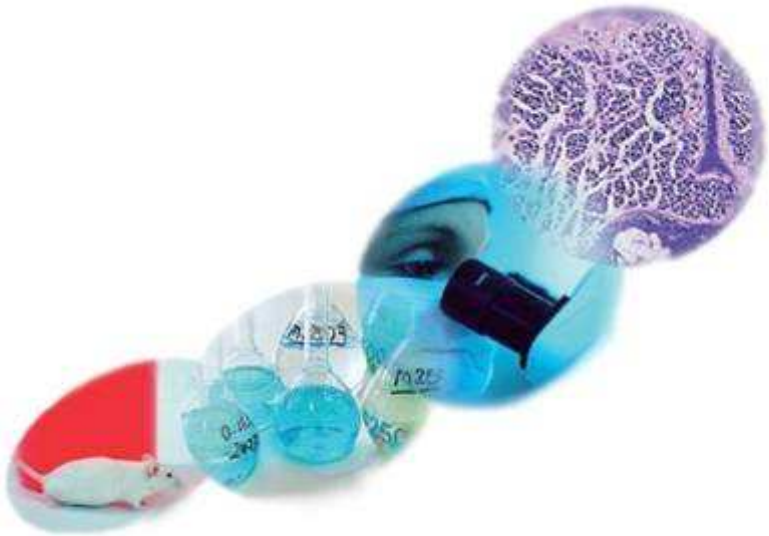


for more information visit

http://ecvam.jrc.ec.europa.eu/

Alternative Methods to Animal Testing:

Improving the Scientific Basis for the Protection of Human Health and the Environment while Reducing the Need for Animal Testing



European Centre for the Validation of Alternative Methods

Additional Slides

Workshop report

- **The Use of Fish Cells in Ecotoxicology. ECVAM Workshop Report 47. Castaño et al. (2003). ATLA 31, 317-351**

Acute aquatic toxicity

- **Threshold Approach (*reduction*)**
  - **proposal as OECD Guidance document**
- **Zebrafish Embryo Toxicity Test (*replacement*)**
  - **coordination of study at OECD level**

48 h pf zebrafish embryo, photo: UBA, D

Bioconcentration/accumulation

- **Validation (Module 1-4) of in vitro trout S9 fraction assay (end in 2010)**
  - **in vitro information on metabolic stability of substances will be used to refine bioaccumulation/bioconcentration models for derivation of BAF/BCF values**

International collaboration

- **HESI, CEFIC-LRi on alternative methods**

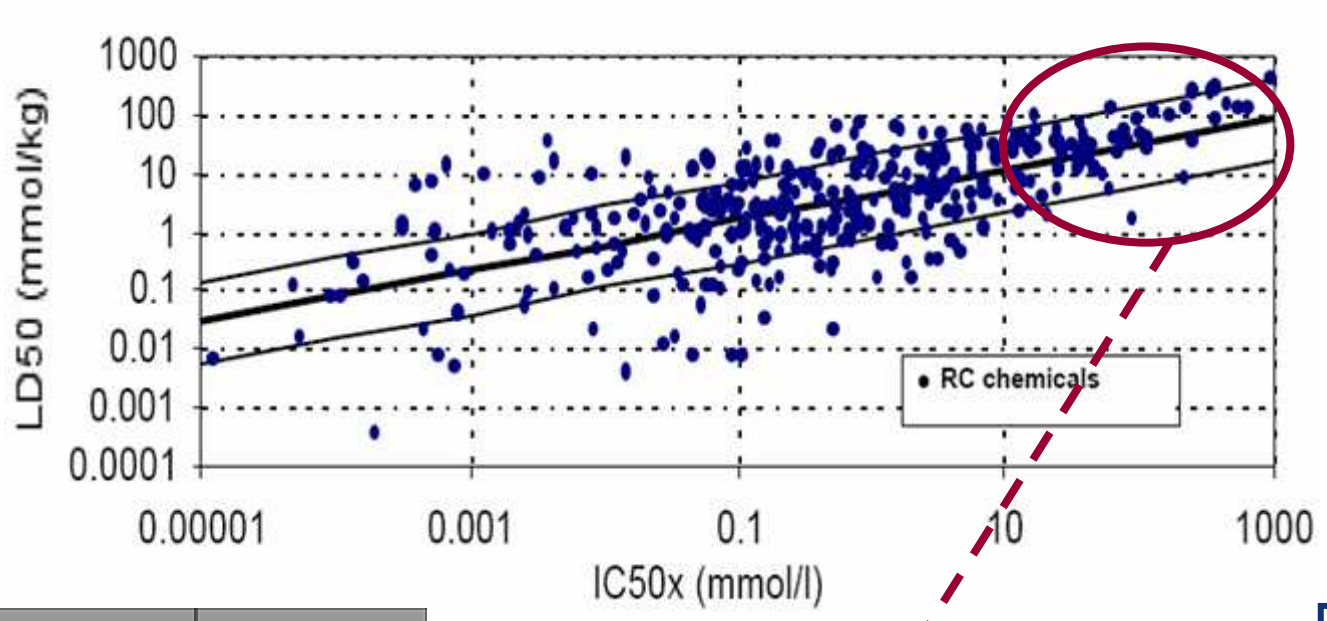
Acute toxicity - Overview main activities

1. Strategy to replace acute oral toxicity testing – FP6 A-Cute-Tox

2. Prediction of non-toxic substances *in vitro* by the Balb 3T3/NRU cytotoxicity test – Validation Study

3. Retrospective data analysis assessing value of testing by multiple exposure routes (Concordance of acute oral, dermal and inhalation toxicity data)

2. ECVAM follow-up 3T3/NRU validation study



From: The Registry of Cytotoxicity
(W. Halle, 2003, ATLA, 31:89-198)

Toxicity class	Prevalence
Category 1 LD_{50} <5 mg/kg b.w.	0.05%
Category 2 LD_{50} 5-50 mg/kg b.w.	0.31%
Category 3 LD_{50} 50-300 mg/kg b.w.	3.41%
Category 4 LD_{50} 300-2000 mg/kg b.w.	9.39%
Category 5 LD_{50} 2000-5000 mg/kg b.w.	70.16%
Not classified LD_{50} >5000 mg/kg b.w.	16.69%

In this class are 86% of all new industrial chemicals

predictive capacity of the 3T3/NRU assay to identify substances with acute oral LD50 > 2000 mg/kg b.w

EU new chemicals database (27/03/2008) – Prevalence acute oral toxicity (4773 substances with oral toxicity data) Bulgheroni et al., 2009

3. Concordance of acute oral, dermal and inhalation toxicity data

Comparison of LD_{50} values/classifications: oral vs. dermal and inhalation

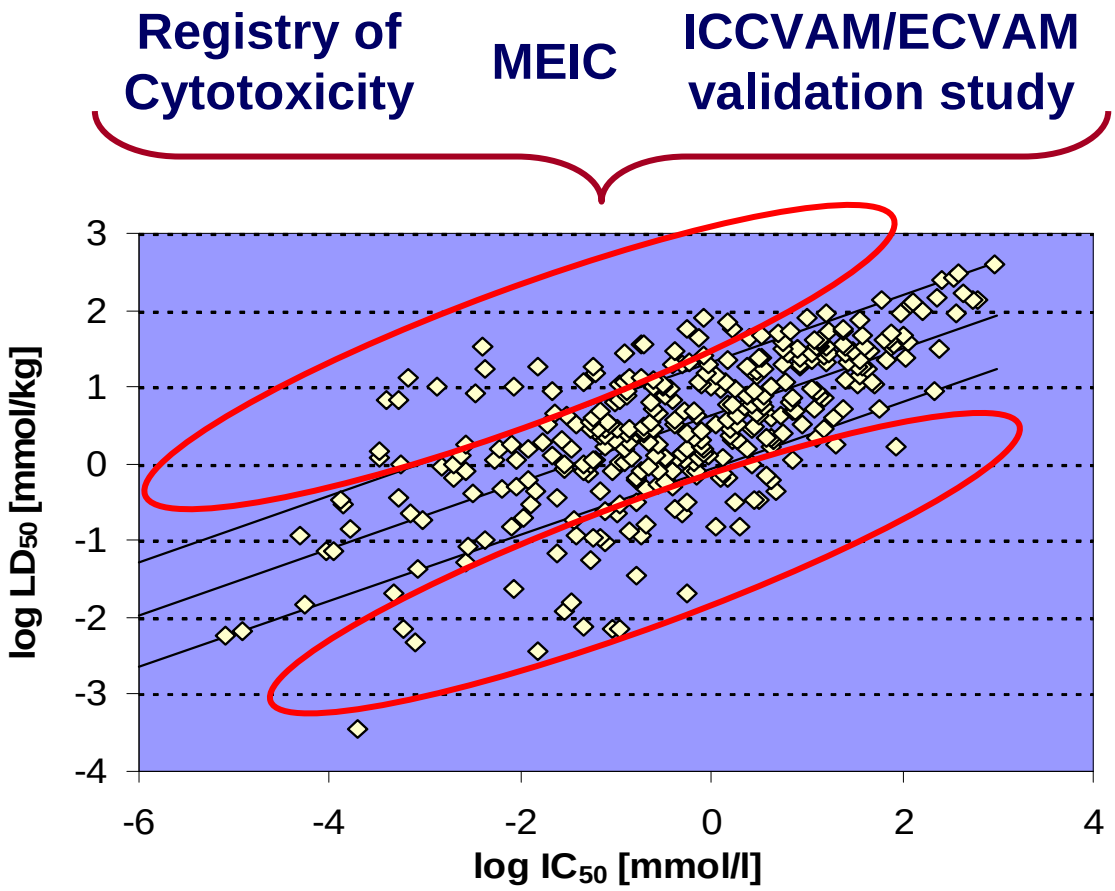
- >1,500 substances from EU New Chemicals Database

- Key findings:
  - Dermal LD_{50} data do not add value for classification & labelling of pure substances;
  - Oral-inhalation concordances require further examination

- Manuscript in preparation for Regulat. Toxicol. Pharmacol.

In collaboration with the EPAA Acute Toxicity Task Force

1. *In Vitro* Strategy to Replace Acute Toxicity Testing



Simple *in vitro* cytotoxicity test:

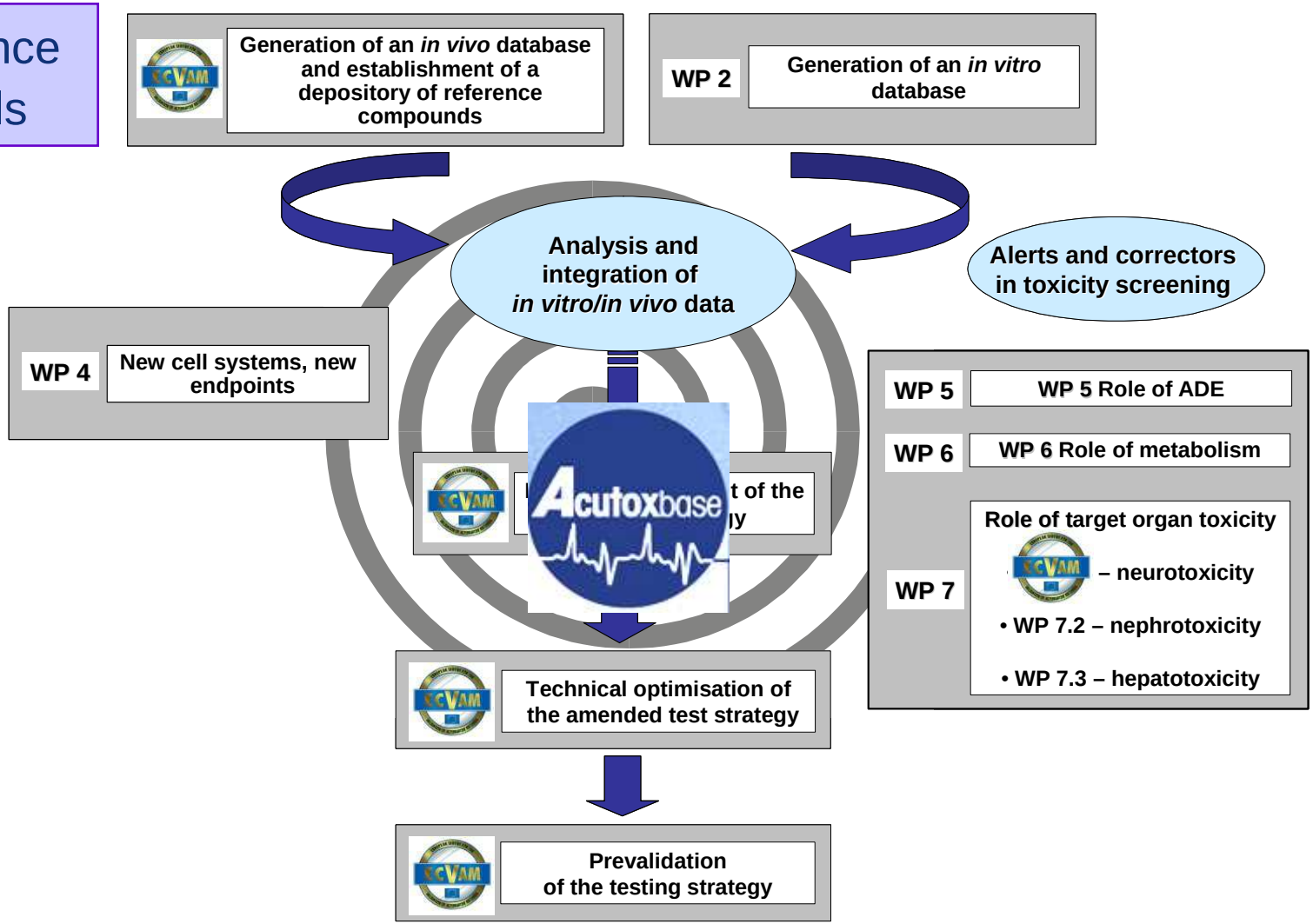
in vitro/in vivo correlation ~ 50-60%

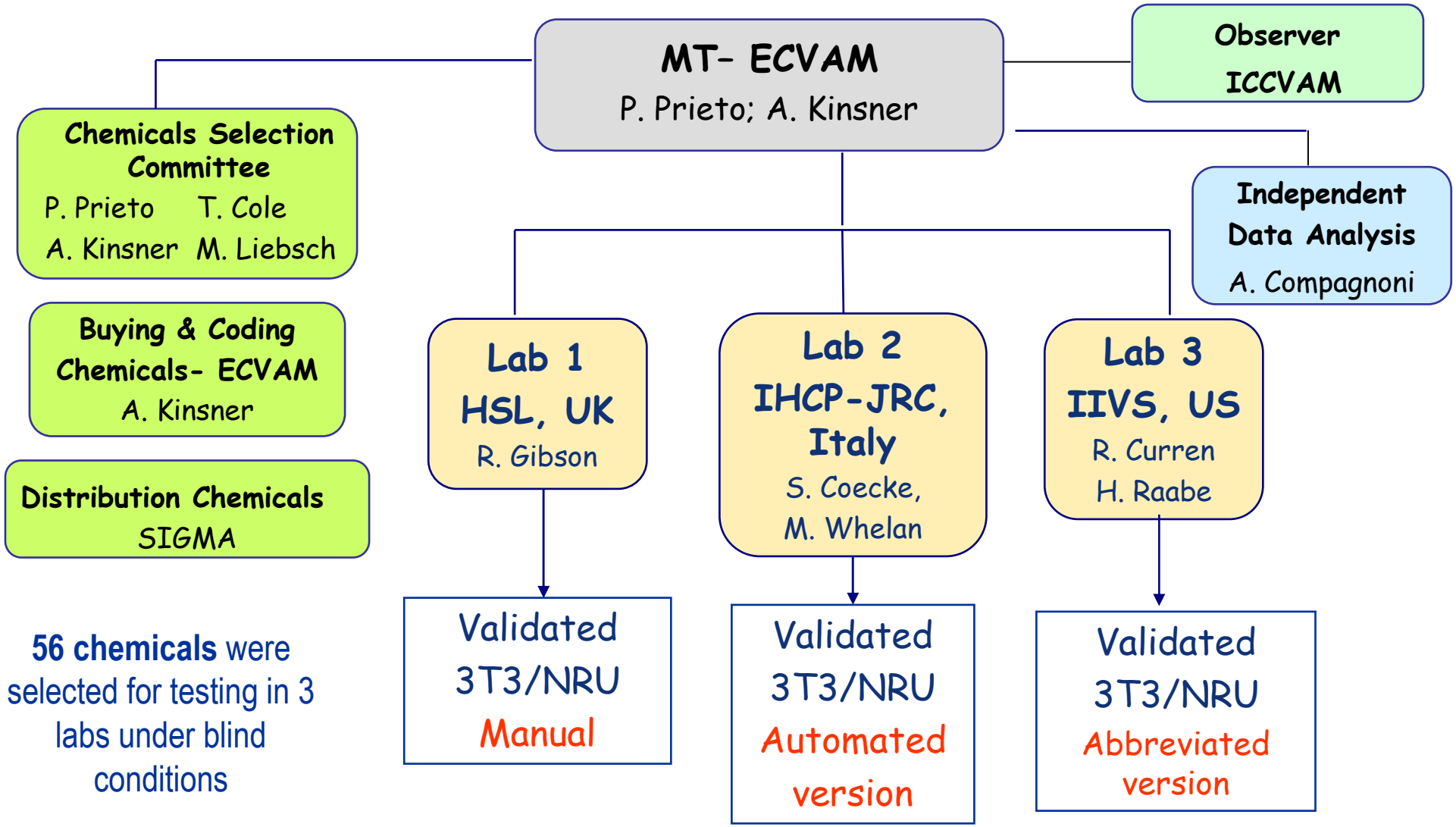
certain number of misclassifications

Further needs:

To improve the *in vitro* - *in vivo* correlation by evaluating existing outliers in order to introduce further parameters (ADE, metabolism, organ specificity).

97 reference chemicals





56 chemicals were selected for testing in 3 labs under blind conditions

Sensitisation: Validation

Validations

Three partial replacement methods for skin sensitisation testing developed by Colipa-associated industries and optimised within Colipa ring trials have entered a Phase III prevalidation for the assessment of their reproducibility and preliminary evaluation of their predictive capacity

- **Direct Peptide Reactivity Assay (DPRA, Procter & Gamble).** Protein binding is a key step in the induction of skin sensitisation, this test uses HPLC to monitor a chemical's potential to deplete a nucleophile-containing synthetic peptide.

- **Human Cell Line Activation Test (h-CLAT, Kao and Shiseido).** This test monitors, using flow cytometry, the induction of two protein markers on the surface of a human monocytic leukemia cell line following exposure to the chemical.

- **Myeloid U939 Skin Sensitisation Test (MUSST, L'Oréal).** This test monitors, using flow cytometry, the induction of a protein marker on the surface of a human dendritic cell like line following exposure to the chemical.

Sensitisation: Research Activities, Sens-it-iv IP

The sixth Framework Programme sponsored Integrated Project Sens-it-iv aims to make available by 2010 a panel of in vitro assays for the identification of skin and respiratory sensitisers for use in the chemical, cosmetic and pharmaceutical industries. ECVAM is leader of WP1 on chemicals selection, involved in WP8 (in vitro assays development) and WP9 (technology transfer and dissemination), in the management and steering of the project.

WP1 *Activities*

Compound selection

The Sens-it-iv list of chemicals comprises 6 respiratory sensitisers, 13 skin sensitisers (including 3 pro-haptens and 1 pre-hapten) and 10 negatives. For the detailed chemical list refer to: http://www.sens-it-iv.eu/index.php?id=701

2. Chemicals Repository

Chemicals are purchased and stored in the ECVAM repository. Aliquots are prepared and distributed to project participants.

3. Guideline for chemical handling

General guidelines on how to handle and dissolve chemicals were developed and distributed to participants.

4. Database of in vivo and in vitro data from the literature

A literature search is performed on a continuous basis to retrieve *in vivo* and *in vitro* data on skin sensitisation

Reduced-LLNA (r-LLNA)

- Retrospective analysis of published data for 211 (169 sensitisers 42 non-sensitisers) chemicals generated with the standard test to explore opportunities to further reduce animals number by limiting the doses necessary to classify chemicals as sensitisers or non-sensitisers
- rLLNA protocol same as standard LLNA protocol but uses only the equivalent of the highest-dose group in the traditional test
- Does not provide information of potency
- For risk assessment purposes a standard LLNA should be conducted
- ESAC statement: April 2007, currently under evaluation its inclusion in the OECD TG 429

ECVAM evaluation

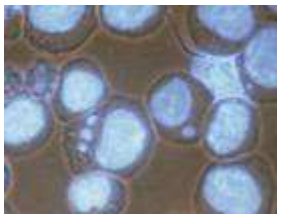
N	Accuracy	Sensitivity	Specificity	False Positive	False Negative
211	98.6 % (208/211)	98.2 % (166/169)	100 % (42/42)	0 % (0/42)	1.8 % (3/169)

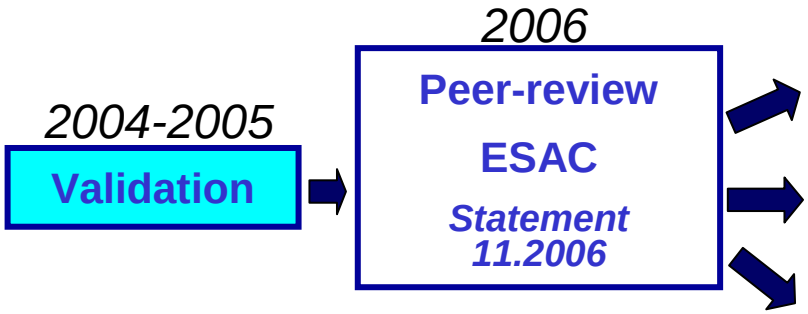
Endpoint Genotoxicity

achievements

Validation

- **Retrospective validation of *in vitro* micronucleus test**





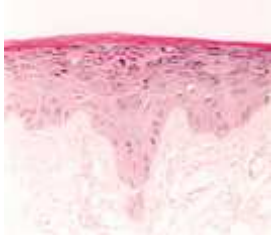
EU Regulatory Acceptance *(REACH 12.2006)*

OECD TG 487 provisionally accepted in 2007 & follow-up with EU/UK group

ICH (*under consideration in revision of guidelines*)

Testing Strategy

- **Workshop on how to reduce false positives in *in vitro* genotoxicity testing (Kirkland et al., Mut Res, 2007)**
- **Recommended chemical list for assessment of the performance of new and improved genotoxicity tests (Kirkland et al., Mut Res, 2008)**
- **Workshop on reduction of animal use in regulatory genotoxicity test (Pfuhler et al, Mut Res, 2009)**



Endpoint Genotoxicity

ongoing activities

Validation

- **Prevalidation of micronucleus and comet assays in reconstituted human skin models**

  (funded by COLIPA and ECVAM and coordinated by COLIPA)

- **Validation of *in vitro* and *in vivo* COMET Assays (coordinated by JaCVAM)**

Testing Strategy

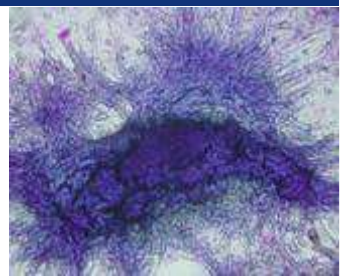
- **Analysis on the top concentration for genotox testing to reduce false positives**

Support research

- **Involved in EC/COMICS project (high-throughput comet assay)**

- **Link with OECD, ICH and IWGT on testing strategy activities above**

Endpoint Carcinogenicity



Validation of three cell transformation assays (CTA)

Focus on reproducibility of standardised protocols

- **OECD recommendation to develop Test Guidelines on CTA (DRP 31)**
- **Experimental work finalised**
- **ESAC peer review (beginning 2010)**
- **Publication of the study in a special issue on CTA of Mut Res, in preparation**

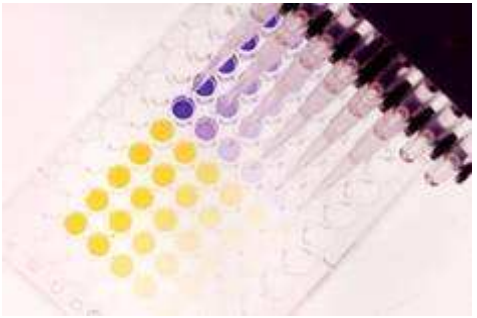
CARCINOGENOMICS FP6 IP

Aim: to develop in vitro toxicogenomics tests to assess carcinogenic potential

- Responsible for workpackage on prevalidation
- Workshop on genomics approaches and cancer risk assessment, Venice, 08/2009

Workshop reports

- **11 workshop reports addressing 3Rs issues in the batch quality control of biologicals (batch safety and batch potency testing)**



Validation & ESAC statements

- **Serological methods (ELISA) for tetanus & erysipelas vaccines (*refinement, reduction*)**
  - *accepted in European Pharmacopoeia monographs*
- **Six in vitro methods based on human fever reaction for pyrogenicity test (*replacement*)**
  - *accepted in Europe & USA*
- **Deletion of the target animal safety test for routine quality control of veterinary vaccines in Europe (no longer relevant)**
  - *currently followed up in collaboration with EMEA at VICH level*

ECVAM/EPAA Workshop

- **The Consistency Approach for Quality Control of Vaccines – a 3Rs opportunity; Brussels, 11-12 January 2010**

Dissemination of Knowledge

- **DB-ALM*:**
  - **Increase in registrations** continues (+- 34 new users/month, Σ1772/75 countries)
  - **Online information content** updating and revisions continued (Annex 1)
  - ***INVITTOX** protocols*:
  - **remote data entry facility** under development
  - **content updating** priority on validated and accepted methods

Complementary activities

- "**ECVAM Guide on good search practices"**
  - Help finding the needed information faster and more efficiently
- **Development of on-line test submission:** Work started
- **Tracking of validation and acceptance status of alternative methods – amending TSAR** foreseen to start later in 2009
- **Portal development** for easier access to listed information systems

***http://ecvam-dbalm.jrc.ec.europa.eu**

Annex 1

DB-ALM Online Information Content

	2009
• ***In vitro* methods**	
◦ Method-Summary Descriptions	139
◦ *INVITTOX* Protocols	130
• Evaluation Studies	45
• Formal Validation Studies	16
• Test Results	8431
• Bibliographic References	5100
• Who's who in the field of alternative methods	202