EUROPEAN COMMISSION
DIRECTORATE-GENERAL
Joint Research Centre

Joint Research Centre

Recent ECVAM Projects Validation & Enlargement Activities

Marlies Halder

IHCP, ECVAM, Ispra, Italy

EUROPEAN COMMISSION
DIRECTORATE-GENERAL
Joint Research Centre

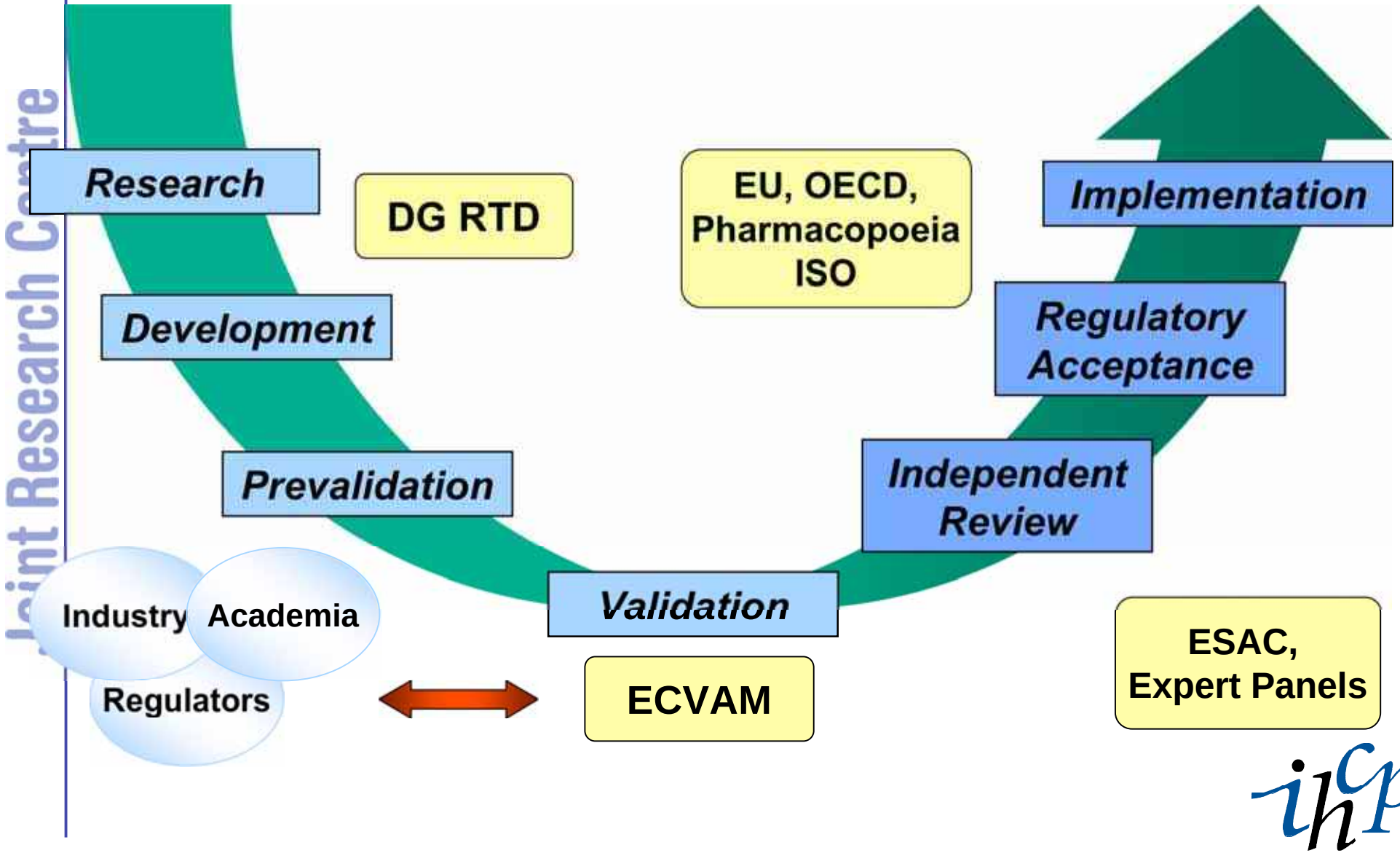
Joint Research Centre

Validation

ihcp

EUROPEAN COMMISSION
DIRECTORATE-GENERAL
Joint Research Centre

Stages in the evolution of regulatory tests



Joint Research Centre

When is formal validation necessary?

… when the introduction of new tests would alter existing legislation.

- EU directives
- OECD guidelines
- European Pharmacopoeia monographs

Regulate the safety testing of chemicals, cosmetics, biologicals,

What is an alternative (replacement) test?

An alternative test can be regarded as the combination of a test system and a prediction model.

ALTERNATIVE TEST	=	TEST METHOD	+	PREDICTION MODEL

A prediction model (PM) is an explicit decision-making rule for converting the results of one or more alternative tests into a prediction of an *in vivo* endpoint.

Major problems in validation studies

- Goals of the study not sufficiently defined
- Studies were poorly designed, planned and managed
- Responsibilities of the participants were not clearly defined
- No SOPs available
- SOPs were not strictly followed

Criteria for test development

For entry into (pre)validation, there should be information on:

1. Scientific purpose and proposed practical application
2. Scientific basis
3. Case for its relevance
4. Optimised protocol, including
   - standard operation procedures
   - endpoints, endpoint measurement, derivation and expression of results, a prediction model
   - the inclusion of adequate controls
5. Limitations/domain of applicability
6. Evidence of reproducibility

Reference: Balls & Fentem (1999). Toxicology in Vitro 13, 837-846.

Prevalidation

Definition:

A PREVALIDATION study is a small-scale inter-laboratory study, carried out to ensure that the protocol of a test method is sufficiently optimised and standardised for inclusion in a formal validation study.

Reference: Curren *et al.* (1995). *ATLA* 23, 211-217

The prevalidation process

Phase I - Protocol Refinement

The protocol and the PM of a test method are refined in a single laboratory with previous experience in the use of the test.

Phase II - Protocol Transfer

An assessment is made of the ability to transfer the method to a second laboratory, making any necessary refinements to the protocol and prediction model.

Phase III - Protocol Performance

The prevalidation process

Actions at the end of Phase III

- **Progress to formal validation**
- **Readiness for incorporation into regulatory guidelines**
- **Further method development is necessary**
- **No further work be undertaken**

Validation

Definition:

A VALIDATION study is a large-scale inter-laboratory study, designed to assess the reliability and relevance of an optimised method for a particular purpose.

Reliability: reproducibility of results within and between laboratories and over time

Relevance: scientific value and practical usefulness

Purpose: the intended application of the procedure

This definition applies to both alternative AND animal methods

The validation process

- Study design
- Selection of tests and laboratories
- Selection and distribution of samples and test reagents
- Data collection and analysis
- Assessment of performance of test(s)

EUROPEAN COMMISSION
DIRECTORATE-GENERAL
Joint Research Centre

ECVAM

Joint Research Centre

Topical Toxicity and Skin Sensitisation

	Development	Prevalidation	Validation	ESAC statement	Regulatory acceptance
Skin Corrosion	✓	✓	✓	✓	✓
Phototoxicity	✓	✓	✓	✓	✓
Skin Irritation	✓	✓	2003		
Eye Irritation	✓	✓	2004		
Skin Sensitisation	✓			✓	✓
Percutaneous Absorption	✓				✓

ihcp

EUROPEAN COMMISSION
DIRECTORATE-GENERAL
Joint Research Centre

Joint Research Centre

Validation of Epiderm & Episkin Assays and Skin Integrity Function Test

Goal:

Replace the Draize skin irritation on albino rabbits EU Annex V, B.4. method, OECD TG 404)

Tests to be evaluated:

The Epiderm and Episkin assays based on reconstituted human epidermis (MTT test)

The Skin Integrity Function Test based on *ex-vivo* mouse skin (barrier function: TEWL, ER)

Skin Irritation Validation Study

EPISKIN	*EPIDERM*	*SIFT*
L'Oréal (F)	ZEBET (D)	Syngenta (UK)
Unilever (UK)	Institute for In Vitro Sciences (USA)	DuPont (USA)
Sanofi-Synthélabo (F)	BASF (D)	TNO (NL)

EUROPEAN COMMISSION
DIRECTORATE-GENERAL
Joint Research Centre

Joint Research Centre

Study Management Team

- **Sponsor:** T. Hartung, V. Zuang
- **Contractor:** Federal Institute fo Risk Assessment (BfR)
- **Lead laboratories:** Dr R. Roguet (L'Oréal), Dr M. Liebsch (ZEBET), Dr J. Heylings (Syngenta)
- **ICCVAM:** Dr. Karen Hamernik (EPA) and Dr. Abby Jacobs (FDA) as replacement, co-chairs of the ICCVAM Dermal Corrosivity and Irritation Working Group
- **NICEATM:** Dr. Bill Stokes (ICCVAM co-chair, NIEHS) and Dr. Ray Tice (ILS, NICEATM senior toxicologist) as replacement
- **Chair of the MT:** Dr P. Botham (Chair ECVAM TF on skin irritation)
- **Co-chair of the MT:** Dr J. Fentem (ECVAM TF on skin irritation)
- **Biostatistician:** S. Hoffmann
- **Chemical selection:** A. Worth and T. Cole

Study design

Phase 1: Preliminary phase

Confirmation of test protocols and prediction models
(20 coded chemicals in lead laboratories)

Training of participating laboratories

Intermediate Analysis

Performance of the assays and comparison with *in vivo* data

Non satisfactory

- Redesign prediction model
- Use supplementary endpoints
- Reconsider strategy and performance criteria

Stop

Satisfactory

Phase 2: Definitive phase

Evaluation of the interlaboratory reproducibility and predictive ability of the tests (3 labs/test; 60 coded chemicals)

Timeframe for completion of the study

January 2004	Start of phase 1/training of participating labs
April 2004	Intermediate analysis of phase 1
June 2004	Start of phase 2
November 2004	Data analysis of phase 2
February 2005	Publication, submission to ESAC

Post-validation

Independent assessment

- Publication of the study in a peer-reviewed journal
- Assessment of the outcome by an independent expert panel
- Statement on the validity by ESAC

Regulatory acceptance

- Draft test guideline
- Submission to regulatory body
- Consultation with expert groups
- Adoption and publication of the new test guideline

EUROPEAN COMMISSION
DIRECTORATE-GENERAL
Joint Research Centre

Joint Research Centre

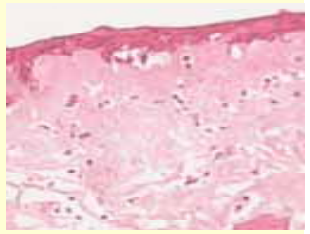
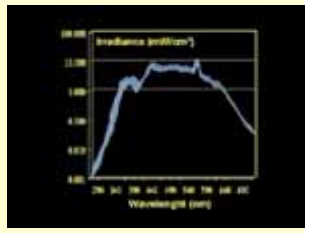
Achievements

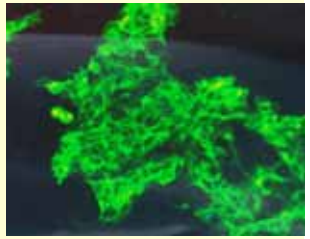
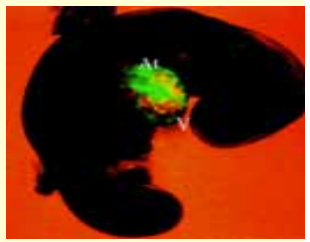
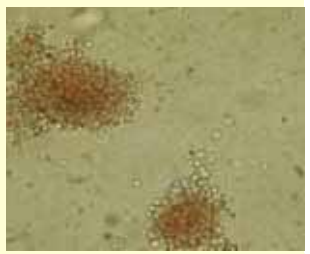
Accepted *in vitro* methods

- Skin corrosion
- Phototoxicity
- Percutaneous absorption
- QC of tetanus & erysipelas vaccines

Validated *in vitro* methods

- Pyrogenicity
- Embryotoxicity
- Haematotoxicity

EUROPEAN COMMISSION
DIRECTORATE-GENERAL
Joint Research Centre

Joint Research Centre

JRC Enlargement Action

ihcp

ECVAM's activities

Aims

- Start and extend collaboration
- Provision and exchange of information
- Provision of training

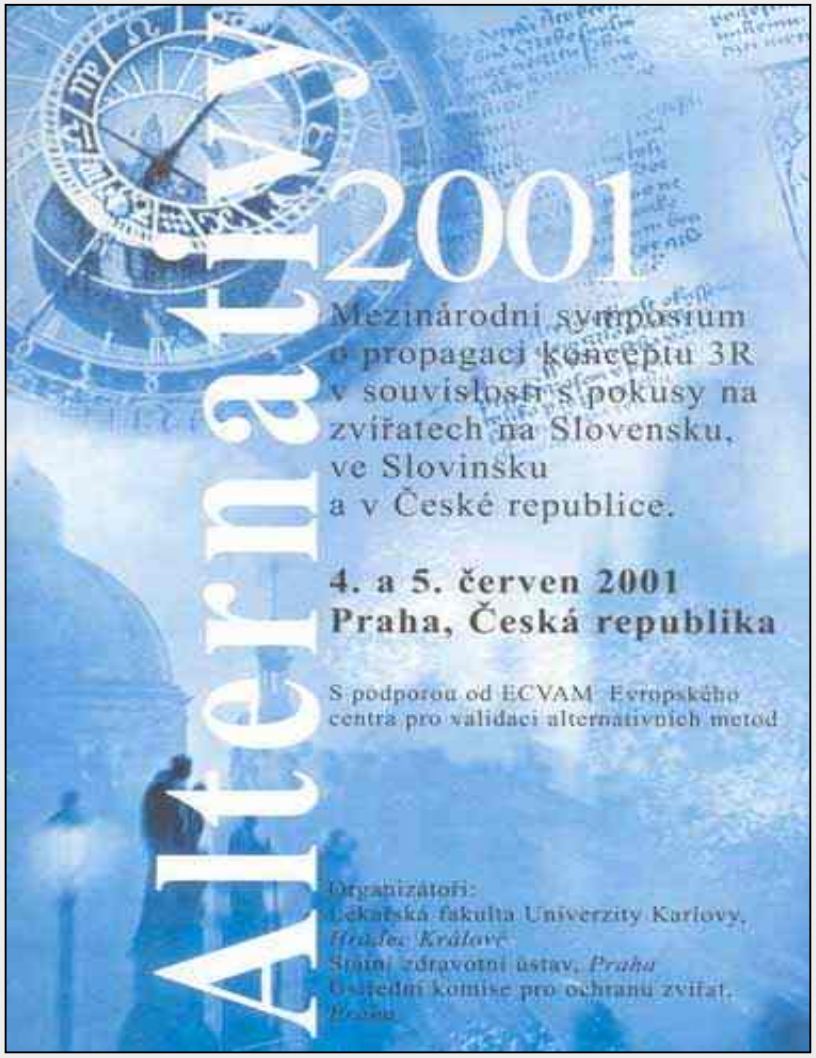
Actions

- Conferences & Workshops
- Training visits (PhD, VS)
- Training courses
- Technology transfer/research

Conferences

- Prague (2001); Warsaw and Budapest (2002)
- General information on ethical and legal background of the Three Rs and *Directive 86/609/EEC*
- Specific information on validation, alternative methods for the testing of chemicals, cosmetics, medical devices, biologicals etc, GLP
- 65 to 100 participants/conference
- Presentations by experts from EU Member States
- Participants from CCs presented their work

Conferences



Workshops

Validation of alternative methods

November 2001 at ECVAM

Alternatives to the use of animals in higher education

April 2002 in Piran (Slovenia)

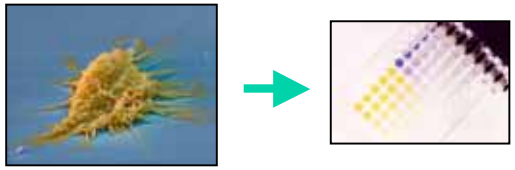
October 2003 in Warsaw (Poland)

The use of QSAR in regulatory testing strategies

October 2002 in Prague (Czech Republic)

- **Up to 25 participants**
- **Experts from MS and CCs presented their work**
- **Discussion forum**

Practical training courses



In vitro methods for pyrogenicity testing

September 2003, Budapest (Hungary)

Quality control of drugs, blood products, medical devices etc

In vitro production of monoclonal antibodies

March 2004, NVI, The Netherlands

Replacement of the *in vivo* production using mice

Training visits

PhD students (2)

- ECVAM research project on neurotoxicity
- Project on QSAR (now ECB)

Visiting scientists (2)

- ECVAM Key Area Systemic Toxicity (Neurotoxicity)
- ECVAM Key Area Reproductive Toxicology

Short training visits (3)

- Specific training on *in vitro* method for embryotoxicity testing in ECVAM's lab

Technology transfer/research

Project: **The use of stem cells and reference models for developmental and chronic neurotoxicity testing *in vitro***

- **National Veterinary Research Institute & Medical Research Centre, Poland**
- **Scientists involved participated in ECVAM's activities:**
  - **Conference in Warsaw**
  - **Training visit at ECVAM**
- **Objective is to optimise promising models for prevalidation**

Activities in 2004

Training courses on Three R methods for the production and quality control of biologicals

- Veterinary vaccines
- In vitro production of monoclonal antibodies

Call for Visiting Scientists and Detached National Experts

- QSARs
- Reproductive toxicology
- Ecotoxicology

Call for PhD students and Post Docs

- to be published on IHCP website

Thank you for your attention!