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Alternative Testverfahren und intelligente Teststrategien
– Position der EU-Kommission –

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Ispra (Va), Italy

http://ecvam.jrc.it

Purposes of animal experiments in 2002

Total number	**10,700,000**	**100%**
Safety evaluations	**1,060,000**	**10 %**
Agricultural chemicals	**123, 000**	**1 %**
Industrial chemicals	**136,000**	**1 %**
Cosmetics	**2,700**	**0.025%**

REACH

30,000 chemicals > 1 t per year to be assessed

Various Estimates:

- ⇨ **Costs:** **2,4 (ECB) – 8 (MRC) billion €**
- ⇨ **Animal numbers:** **3,9 (ECB) – 43 (BfR) million**
- ⇨ **Time foreseen:** **11 (ECB) – 40 (MRC) years**

Calulated savings up to 70% of costs and animal numbers, if intelligent testing strategies are applied (i.e. read-across, QSAR, in vitro, refined in vivo).

billions of Euro, millions of animals, decades of testing

The ECVAM Strategy

- **Analyse the animal test >>> REFINE, BENCHMARKS**
- **Analyse the prevalence of health effects >>> TEST STRATEGIES**
- **Analyse what is available >>> INVENTORY**
- **Coach development of tests needed >>> INTEGRATED PROJECTS**
- **Optimise validation process >>> MODULAR APPROACH**
- **Ensure Quality >>> GLP /GCCP**
- **Plan strategies >>> 400 EXPERTS**
- **Bundle all stakeholders >>> INTERNATIONAL COLLABORATION**

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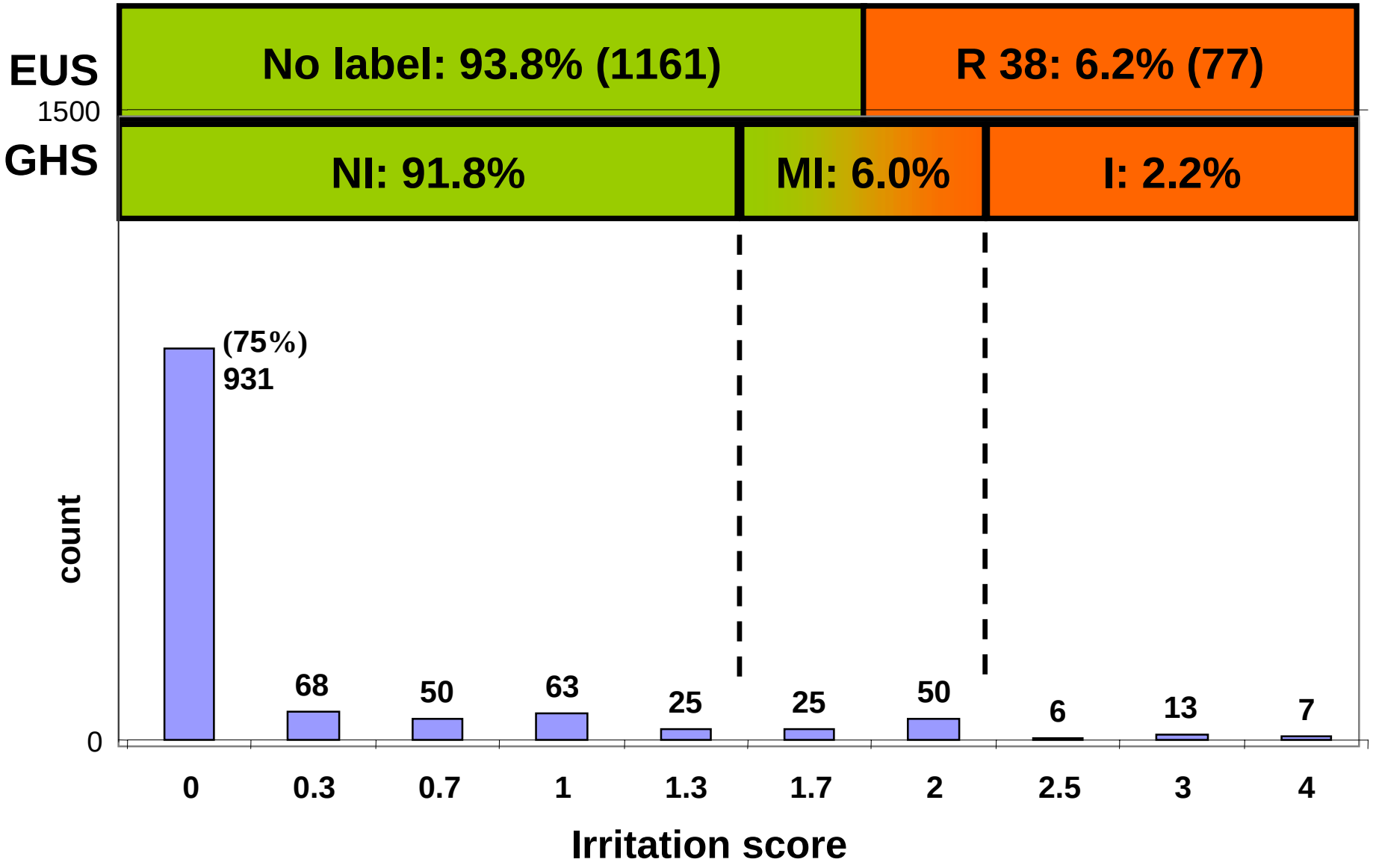
Ecotoxicology

Reduction alternative – Threshold (step-down) approach

- **2003: 1.6 Mio fish used - increase of 970,000**
- **REACH: estimates up to 4 Mio**
- **Retrospective analysis of ecotoxicological data of chemicals and plant protection products (ECVAM & ECB)**
- **Possible reduction of 55-70% in number of fish used**
- **Peer review 2005**

Skin Irritation

NCD-Prevalence (n = 1238)



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)

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EYE IRRITATION in vivo

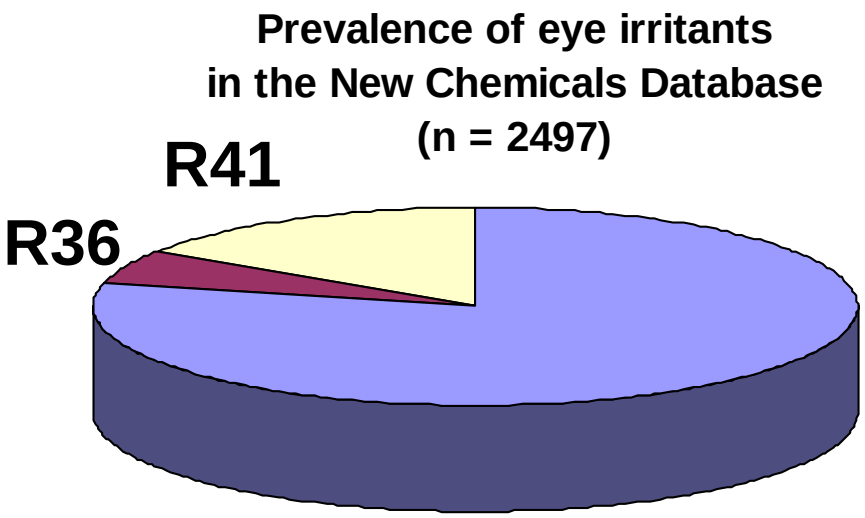
- **5 – 15% underprediction as inter-animal variance within a single test**
- **< within-laboratory variance (day-to-day variances, performers)**
- **< between-laboratory variance (laboratory set-up, animal strains, performance)**
- **< predictive capacity for human health effect**

No comprehensive analysis of high-quality data exists;

Review in preparation

→ **our tools are not perfect**

The prevalence concept



- **Negative predictive value R41:**
  85% accuracy: 97%
- **Positive predictive value R41:**
  85% accuracy: 51%

→ **Every test (in vivo or in vitro) will have many false-positives and few false-negatives**

→ **Identify the negatives**

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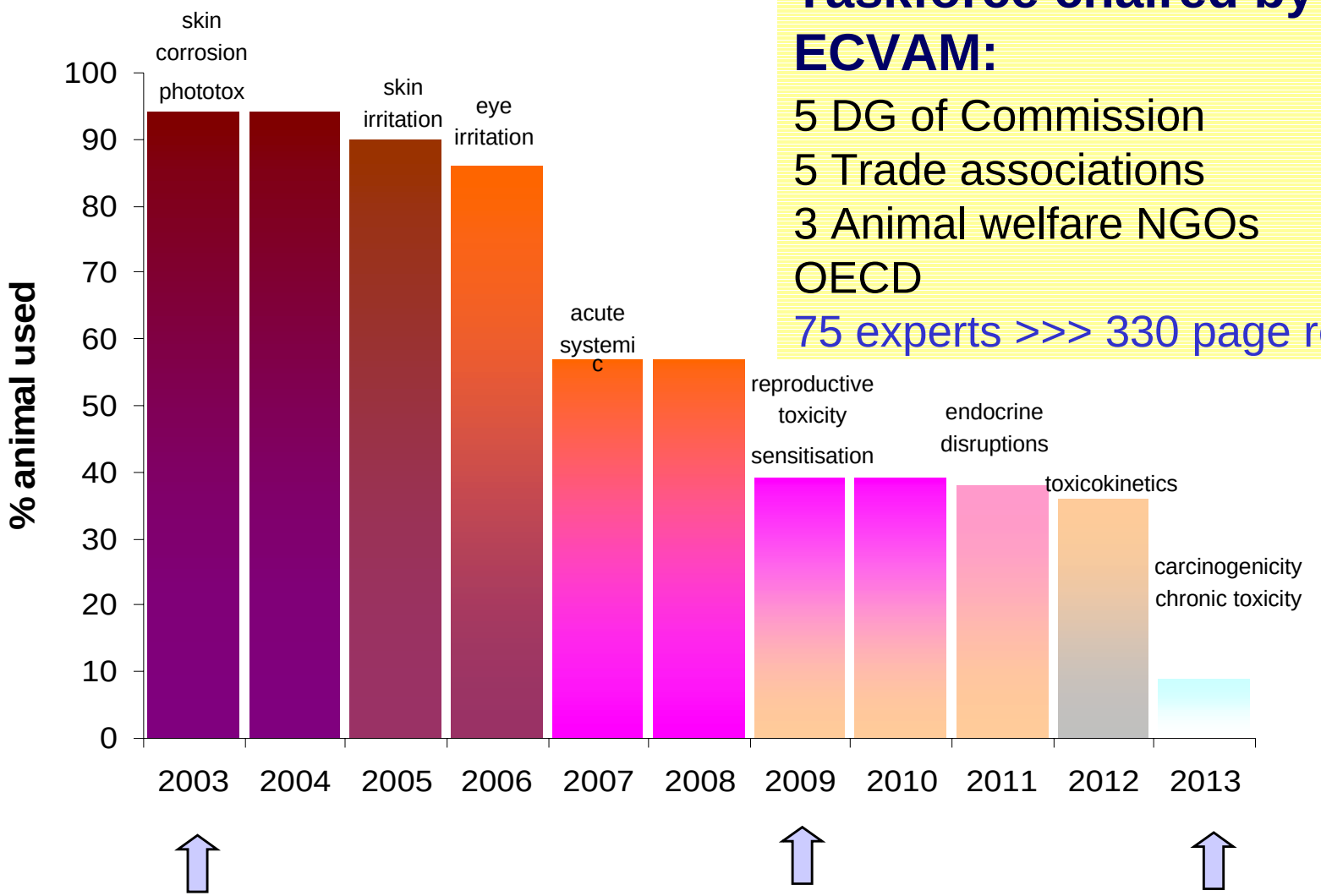
Vision 7th Amendment of Cosmetics Directive



Taskforce chaired by ECVAM:

5 DG of Commission
5 Trade associations
3 Animal welfare NGOs
OECD
75 experts >>> 330 page report

Stop finished products & ECVAM-validated alternatives

Deadlines ingredients

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ECVAM DATABASE SERVICE ON ALTERNATIVE METHODS TO ANIMAL EXPERIMENTATION

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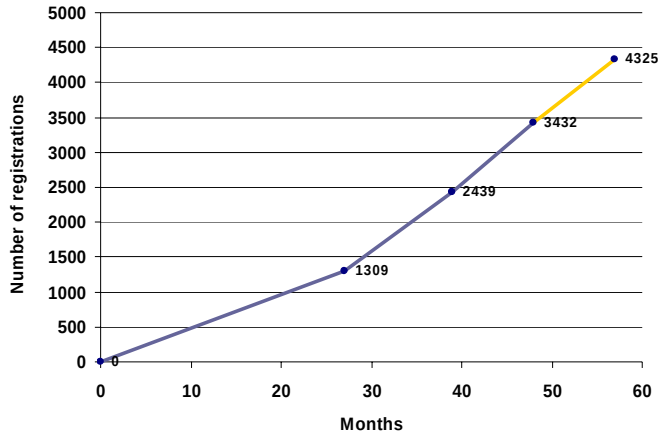
Registration Tendency 2001-2005

4325 registered users from 65 countries

May '05



Data content

In addition to the existing information content, 2 new sectors:

- *In vitro* Neurotoxicity
- *In vitro* Metabolism-mediated Toxicity

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The new dimension of development of alternative methods

DG RTD

DG JRC
ECVAM

Policy-DGs
ECB (DG JRC)

Collaboration in 3 Integrated Projects

(about 90 partners & 30 million Euro)

“ReProTect”, “A-Cute-Tox” & “Sens-it-iv”

Acute toxicity (LD_{50} test)

37% of all animals in toxicology (2002)

- **Seventies** — **150 animals per substance**
- **Eighties** — **harmonised OECD: 45 animals**
- **Nineties** — **tiered testing strategies: 15 animals**
- **Ongoing** — **ECVAM / ICCVAM study: 5 animals (?)**
- **Starting** — **A-Cute-Tox: no animals (?)**

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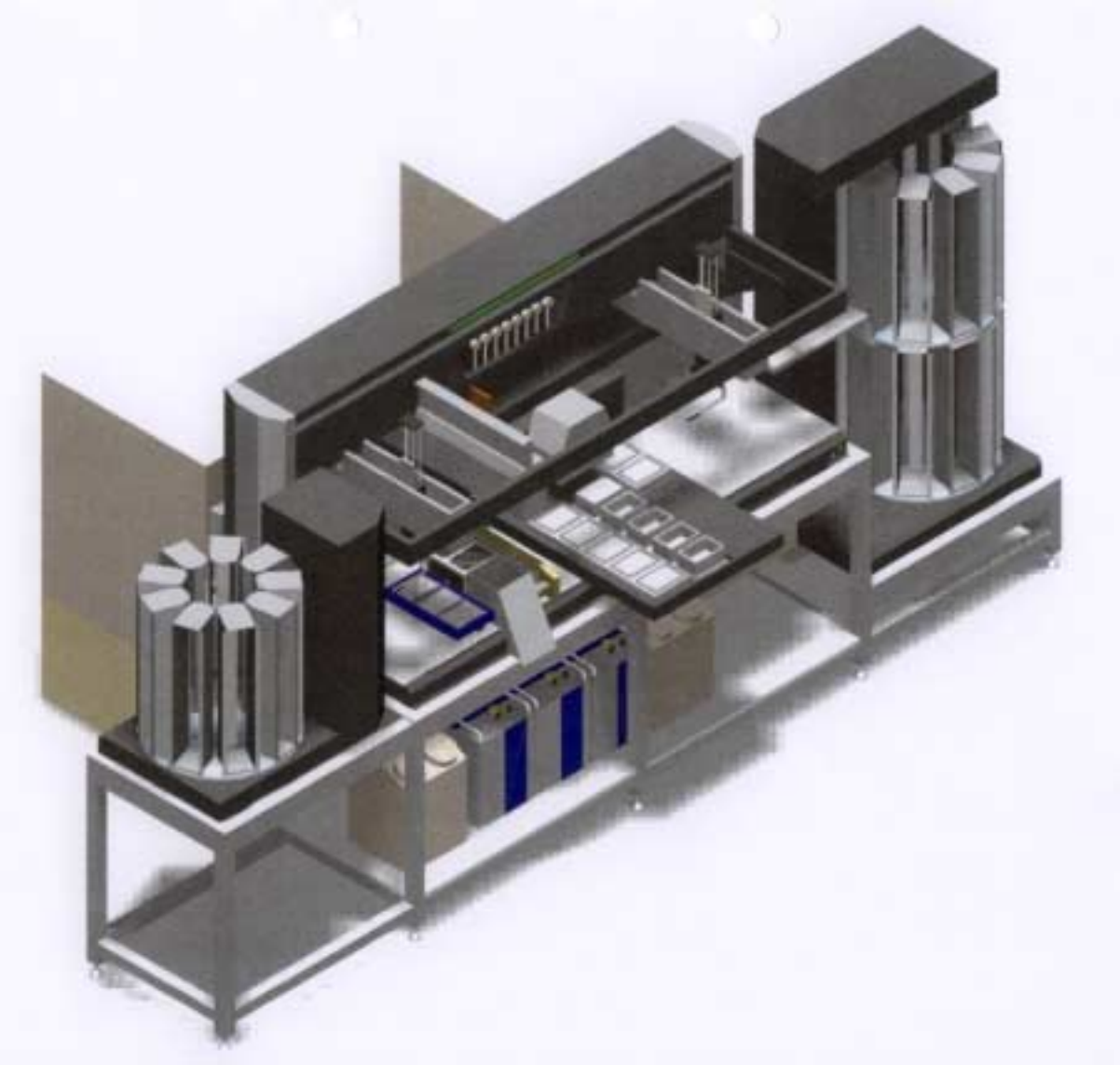
Acute Systemic Toxicity

A-Cute-Tox

Integrated Project
37 partners (14 states), granted 9 M€
17 universities, 10 SME, 2 industry, 6 institutes
Coordinator:
Expertradet, Sweden

ihcp

New concept: Reversed high-throughput



Pharma:

1 million subst.

1 valid cell model

ECVAM:
300+ substances

Variation of cell models

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Reproductive Toxicology

Integrated Project
26 partners (9 states), granted 9 M€
9 universities, 5 SME, 3 industry, 7 gov.
Coordinators:
Univ. Tübingen / ECVAM

Sensitization (Skin & Lung)

Sens-it-iv

Integrated Project

31 participants (11 states), 10 Universities, 6 research institutes, 6 Industries, 7 SME, 1 foundation, JRC

Coordinators

Novozymes / MPI Freiburg

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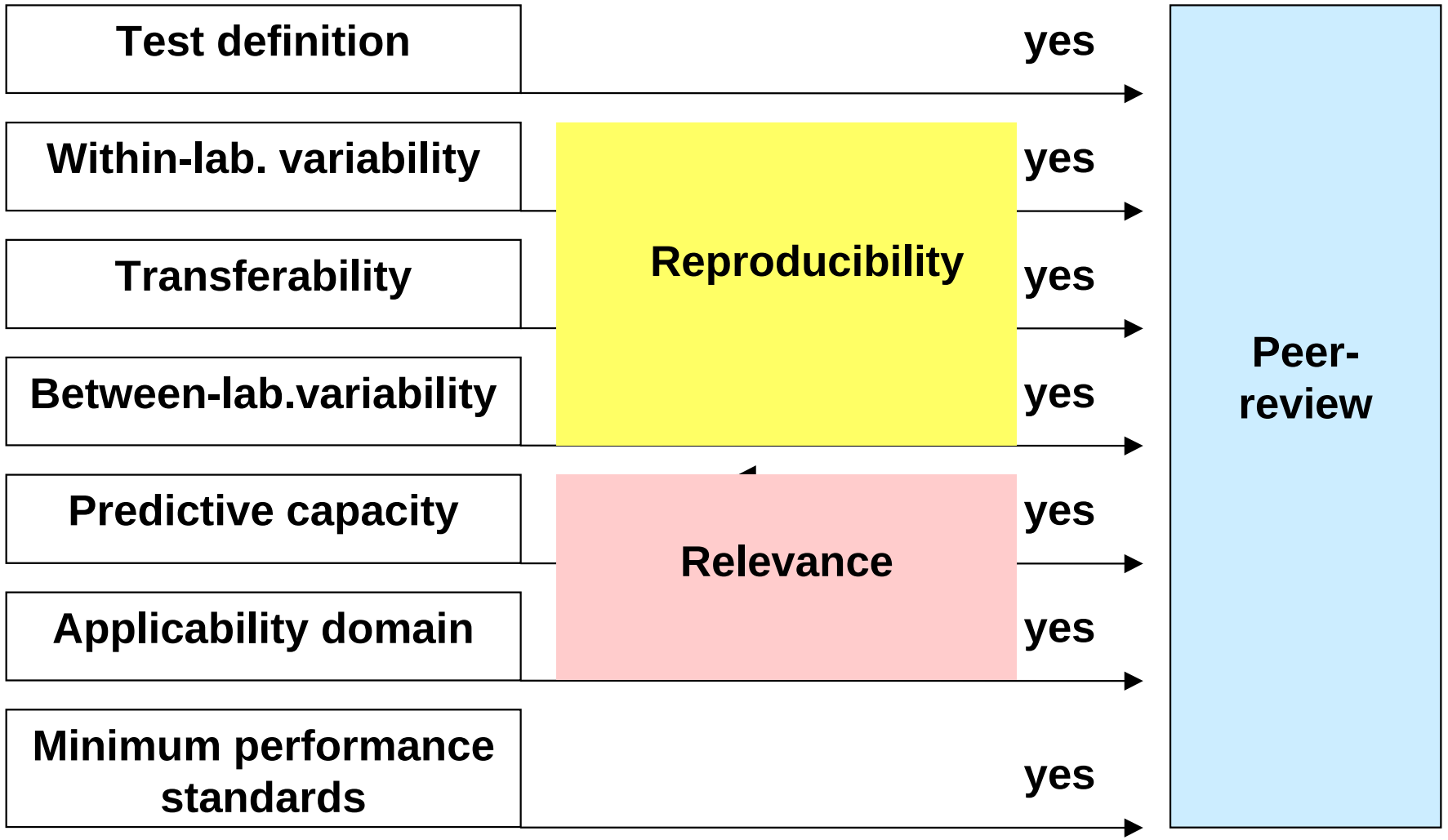
Challenges to the validation process

- **Efficiency of the process** → **reduced testing**
- **Use of existing data** → **weight-of evidence retrospective valid.**
- **New technologies (toxicogenomics, transgenics, (Q)SAR)** → **adaption of principles**
- **Amendments of validated tests** → **incremental validation**

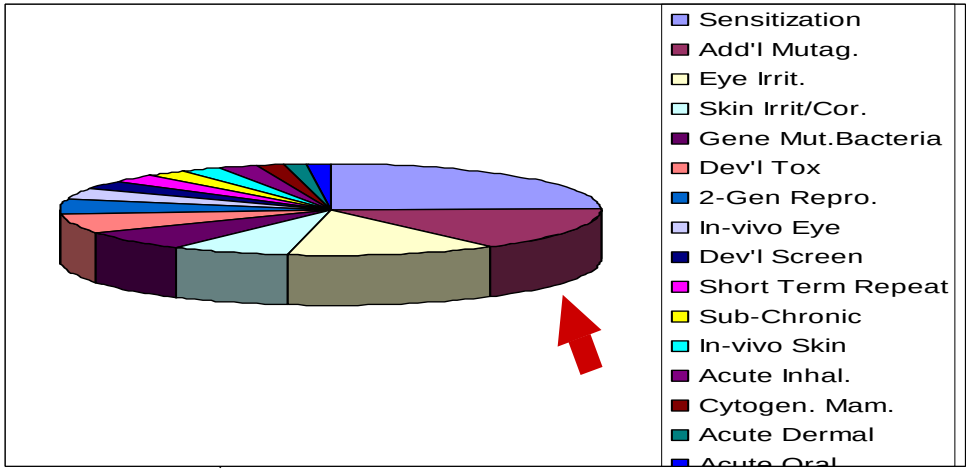
Modular Approach to Validation

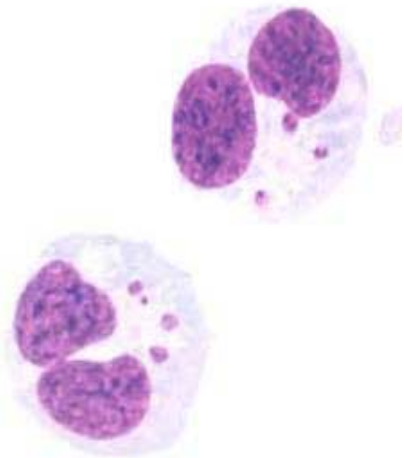
Hartung, et. al. ATLA 32, 467-472, 2004



Mutagenicity/Genotoxicity

2nd largest testing requirement





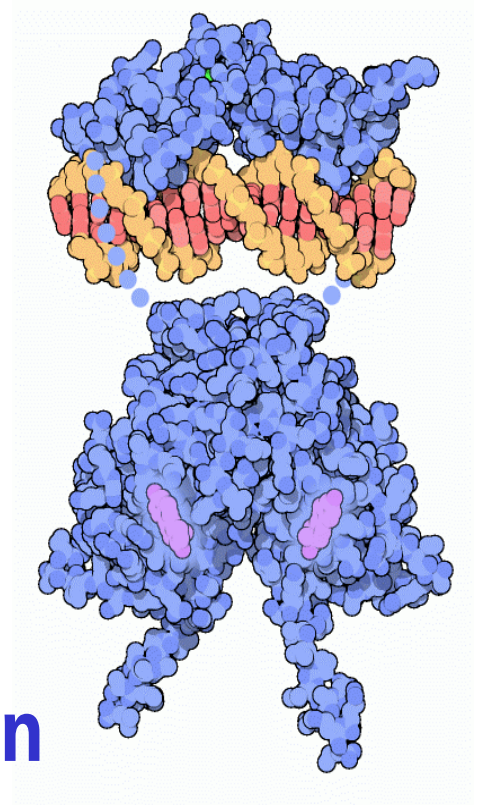
Micronucleus Test in vitro

- 1st retrospective validation
- Peer review foreseen summer ’05
- Parallel OECD activity

ECB/ECVAM work on (Q)SARs

- **External contracts**
  Acute fish toxicity, Skin penetration, Skin sensitisation, Nuclear hormone receptor binding

2. **Collaborative studies**
   Skin sensitisation, Acute local toxicity



Contribution to OECD activities

- **Principles of (Q)SAR validation**

2. **(Q)SAR terminology document**
3. **Guidance Document on (Q)SAR validation**
4. **Guidance on Chemical Categories**

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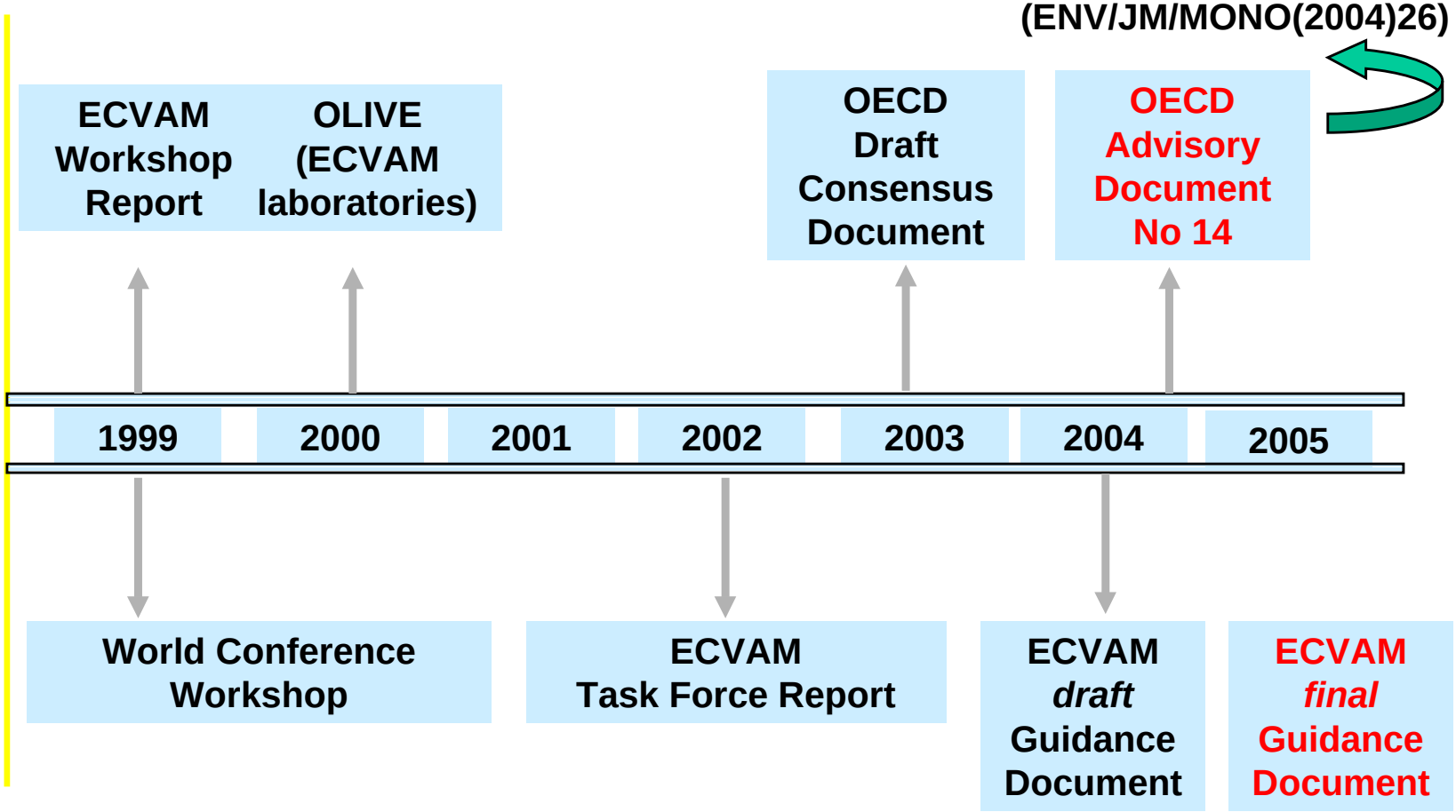
GLP and GCCP

Joint Research Centre

GLP

GCCP



OECD Advisory Document No 14 "The Application of the GLP Principles to in vitro studies" (ENV/JM/MONO(2004)26)

Coecke et al (2005)
Guidance on Good Cell Culture Practice;
ATLA, July issue

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Components of Intelligent Testing Strategies

(i) *In-vitro* tests
(ii) Optimised *in-vivo* tests
(iii) Thresholds of toxicological concern
(iv) In silico (SARs, QSARs, modelling)
(v) Read-across and chemical categories
(vi) Exposure assessment/exposure-based waiving

Eye Irritation

Evaluation of 10 methods (with ICCVAM)

Organotypic methods

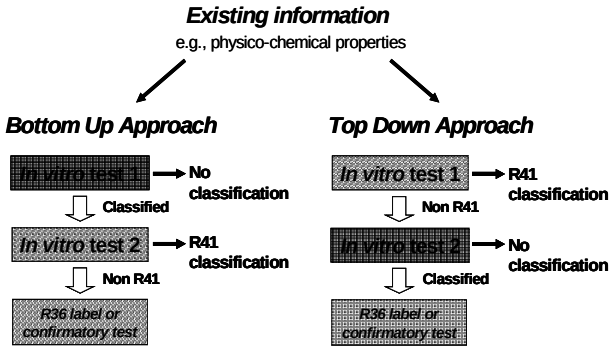
Reconstituted human tissue models

Cell cytotoxicity- / function- based methods

Identification of Testing Strategies

Eye Irritation Expert Meeting, Feb 05

Evaluation of collected data



R36: Irritating to eyes; R41: Severe eye irritant

Review of the in vivo Draize Eye Test

Based on literature

Evaluation of existing data

Other activities: *Validation of LVET (refined in vivo)*
Ocular Toxicity Mechanistic Symposia, May 05 (with ICCVAM)

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Eye Irritation Expert Meeting

Feb. 8-11, 2005

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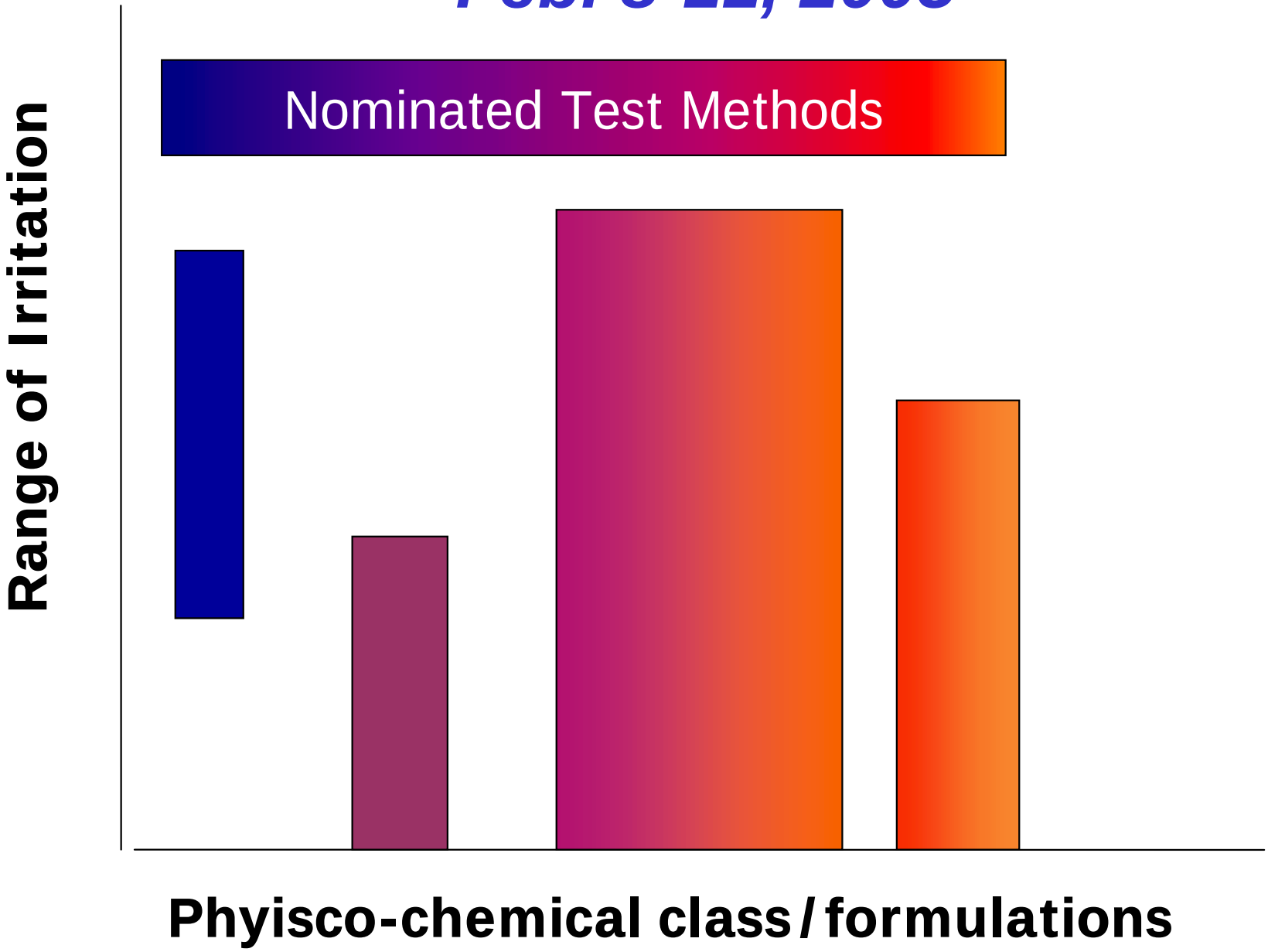

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ECVAM Collaboration
Striving for International Harmonization

ICCVAM

- Joint workshops and studies
- Validation of toxicogenomics
- GLP
- Mutual observer status
- Harmonisation of peer-review process
- Consultation group between ESAC and ICCVAM
- Joint submissions to OECD
- Personnel exchange

OECD

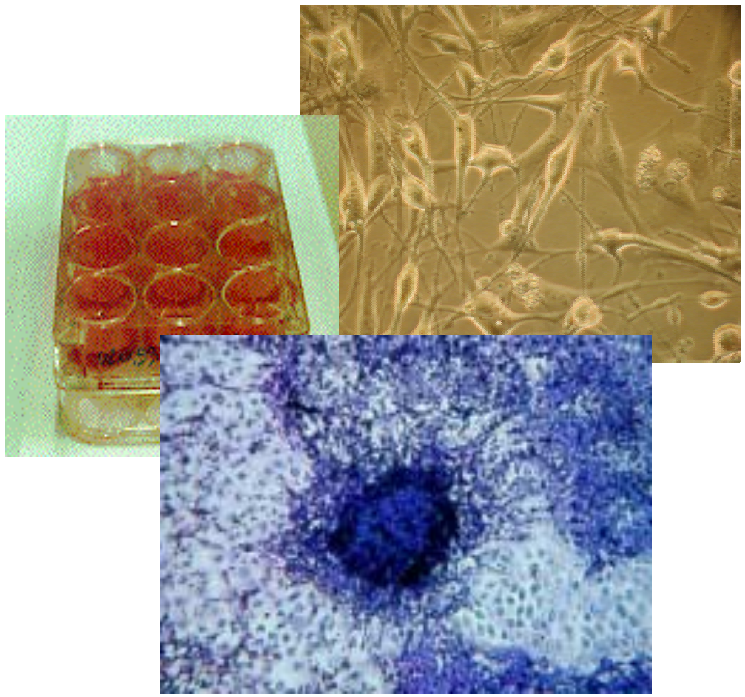
- Secondments
- Observer status on ESAC
- Document 34 on validation
- GLP in vitro guidance document
- Collaboration in validations
  - endocrine disrupters
  - micronucleus test
  - cell transformation assay

Carcinogenicity

The only regulatory accepted test:

2 years animal test (1 M€/chemical)

Alternative: *in vitro* Cell Transformation Assa



Prevalidation initiated in 11/2004

1st study involving EU, USA, Japan

Nothing is as strong as an idea whose time has come.

Victor Hugo