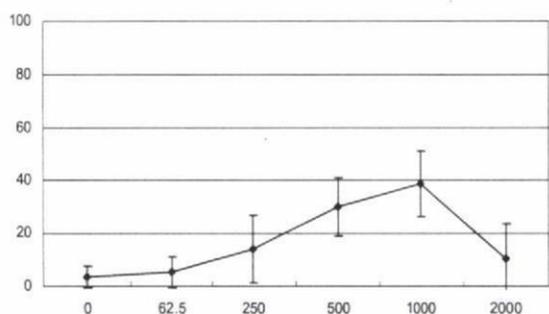
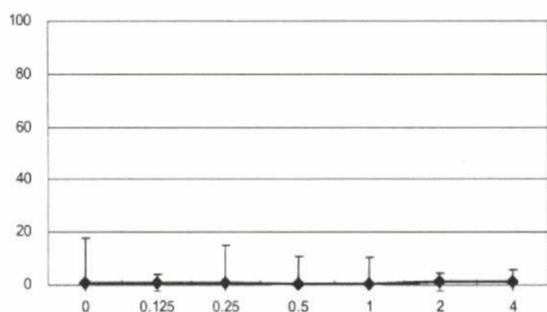


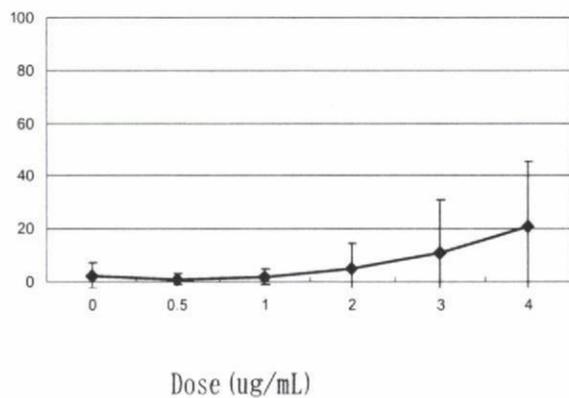
(A) Ethylmethanesulfonate (EMS)



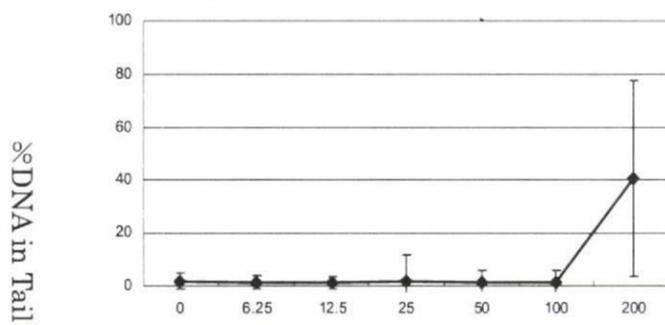
(B) Mitomycin C (MMC)



(C) 2-Aminoanthracene (2-AA)



(D) Cycloheximide



(E) Trinton X-100 (TX100)

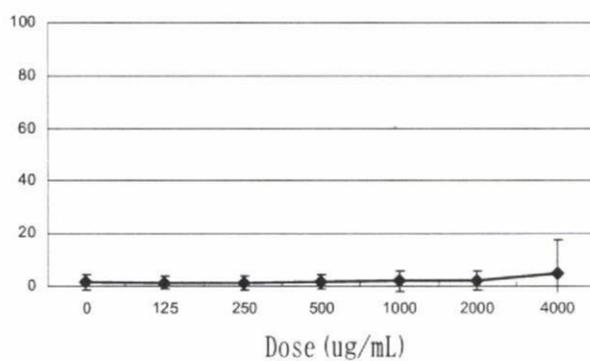


図2. In vitro 試験結果 (リードラボ)

D. 考察

In vivo 試験においては、施設間でバラツキが生じ、陽性対照で適合結果が得られない施設がでた。被験物質においても陽性が予測されたアクリルアミドをすべての施設が評価できないとともに、各施設の反応性が低かった。

原因は定かではないが、陽性対照の投与回数が守られず、また、電気泳動条件も見直さなければならぬ状況である。もう一度、各ラボのヒストリカルデータの検証を行い、細部の詰めを行った後にプロトコルの改良が行われる予定である。

しかし、5施設はそれぞれがコメットアッセイの専門施設であり、これまで施設毎のノウハウが蓄積されている。これが陽性対照のバラツキおよび被験物質の判定結果の違いに結びついていると考える。

また、試験計画もやや意図に欠けたかもしれない。陽性対照の濃度設定や被験物質の選定や濃度設定が妥当であったかの検証は、Phase III以降のバリデーションへの教訓として生かされなければならないと考えている。

国際バリデーションの問題点は、1) 技術講習会を避け、紙面のみで技術の共有化を図ること、2) 熟練した施設が参加する場合、手技が確立されており、プロトコルによる新たな提案を素直に受け入れない点にある。今後、この点を重視したバリデーション研究を構築する必要があると感じている。

これらの改良を受け、Phase IIIのバリデーションとして、5施設に2~3コード物質を配布して追加実験を行い、プロトコルの改善を確認することになった。このバリデーションは5月~8月までの間に実施され、その結果を今秋に開催予定の第5回バリデーション実行委員会で確認することが内定した。

方法および結果には記載しなかったが、Phase IVの多数施設のバリデーション開始に向けて、世界的に参加施設の募集を掛け、約15施設(海外10施設および国内5施設)から参加の申し出を受けている。これらの施設にEMSによるヒストリカルデータの提出を促すとともに、Acrylamideおよび2,6-Diaminotolueneを送付して実験を依頼している。期限は5月末である。これらの結果を待って、参加施設を現在の5施設から15施設まで増やし、年末から開始予定の大規模なPhase IVバリデーション研究に備える予定である。

実行委員会の大きな仕事はこの施設の選別ではなく、Phase IVバリデーションに用いる被験物質の選択である。この大きな課題に向け、早急に実行委員会のメンバーでコンセンサスを取る必要がある。現在、宇野 in vivo 委員長が目的にあった10のカテゴリーを選別し、それに適合する化学物質リストの作成を進めている。

なお、動物数の削減に関する議論は重要であるが、本年度の実行委員会では議論する余地がなかった。来年度以降の課題である。

一方、in vitroについては、Phase Iバリデーションを遂行中である。ただし、現時点ですでにプロトコルの問題点が提起され、Phase IIバリデーションの追加実施が決まっている。こちらも年内に早急にプロトコルを確定し、2009年早々からPhase IIIバリデーションを開始する予定である。

E. 結論

In vivo 試験においては、Phase IIバリデーション研究において施設内間でバラツキが生じ、陽性対照で適合結果が得られない施設がでた。

In vitro 試験においても、Phase Iバリデーション研究を遂行中である。現時点ですでにプロトコルの問題点が提起され、Phase IIの追加バリデーション研究の実施が決まっている。

F. 健康危険情報 なし

G. 研究発表

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H. 知的財産権の出願、登録状況
なし

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J. 添付資料

- 添付資料1: JaCVAM ワークショップ資料 ECVAM The European Centre for the Validation of Alternative Methods: Mission and Organisation
- 添付資料2: JaCVAM ワークショップ資料 JaCVAM (The Japanese Centre for the Validation of Alternative Methods) Mission and Organisation
- 添付資料3: JaCVAM ワークショップ資料、OECD テストガイドラインの概要、手続き、規制における活用、最近の動向
- 添付資料4: JaCVAM ワークショップ資料 The Comet Assay - an overview
- 添付資料5: JaCVAM ワークショップ資料 *In Vivo* Comet Assay: Update on the On-Going Validation
- 添付資料6: JaCVAM ワークショップ資料 In Vivo Alkaline Comet Assay: Room Temperature Compared to 4° C Unwinding and Electrophoresis; 2 mM EDTA Compared to 10 mM EDTA in Gel
- 添付資料7: JaCVAM ワークショップ資料 Validation of *in Vitro* Comet Assay, -Pre-validation to make a robust protocol and consensus for evaluation and interpretation-
- 添付資料8: Protocol International Validation of the in vivo Rodent Alkaline Comet Assay for the Detection of Genotoxic Carcinogens (Version 13)
- 添付資料9: Study plan
- 添付資料10: Fig. and table
- 添付資料11: International Rre-validation study of the in vitro Alkaline comet assay by Dr. Honma
- 添付資料12: Protocol International Pre-Validation study of the in vitro

Alkaline Comet Assay (Version4.3)

添付資料 13 : Draft Minutes The 1st
International in vitro comet assay
validation study

添付資料 14 : Draft Minutes The 3rd
International Validation Meeting on Comet
Assay

添付資料 15 : Draft Minutes The 4th
International Validation Meeting on Comet
Assay



JRC
EUROPEAN COMMISSION





ihcp
Institute for Health and Consumer Protection

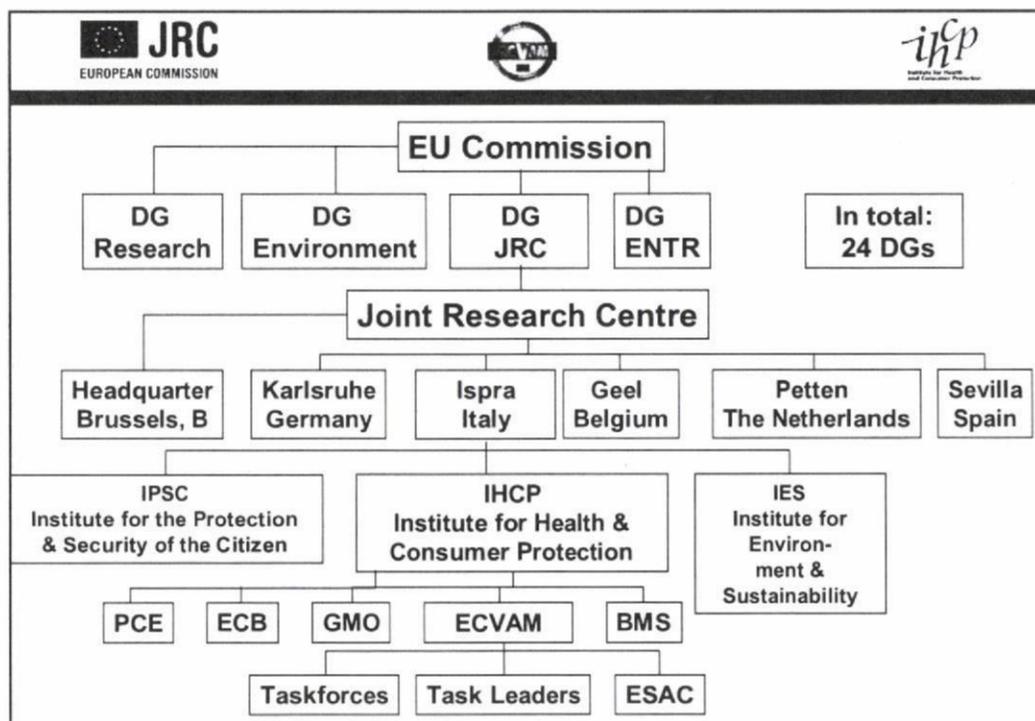


ECVAM

The European Centre for the Validation of Alternative Methods: Mission and Organisation

Raffaella Corvi and Thomas Hartung

Workshop on Comet assay, NIHS, Tokyo, 11 March 2008



European Centre for the Validation of Alternative Methods ECVAM



- Validation
- Research
- Database
- Communication

EU Policy support

- 7th Amendment to
Cosmetics Directive
- REACH

Cosmetics industry and the 7th amendment

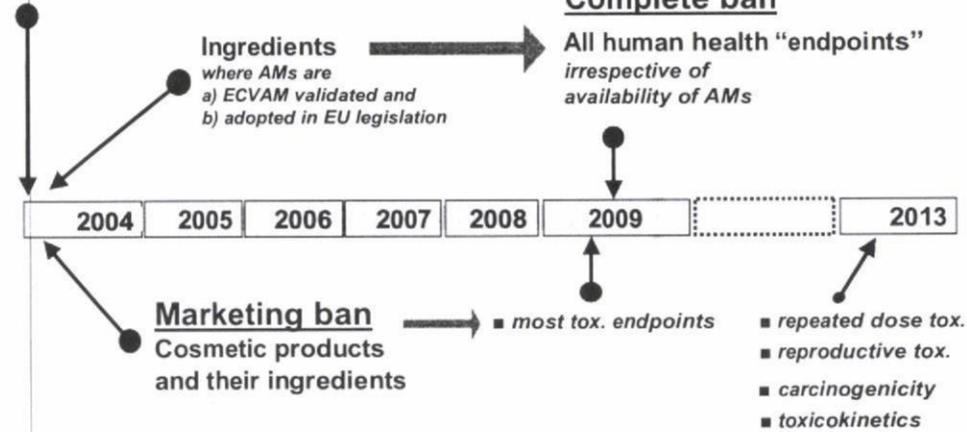


- EU: 2000 companies, 60 billion € turnover
- EU: 5000 new products per year
- 25% turnover due to products released within last 6 months
- Phasing out animal testing in ingredients by 2009 and marketing bans by 2009 and 2013
- Critical need for alternatives

7th Amendment to Cosmetics Directive

Ban on animal testing

Cosmetic products



EU Chemical legislation: REACH

(Registration, Evaluation, Authorisation of Chemicals)

Investment into safety

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

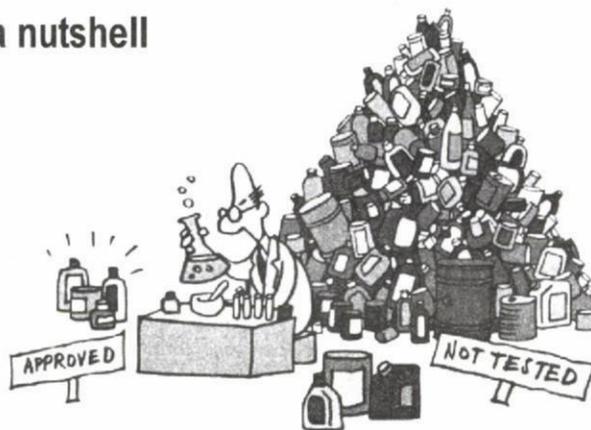


billions of Euro, millions of animals,
decades of testing



Major impact of *in silico* and *in vitro* tests

REACH in a nutshell



picture
© ChemSec

ECVAM involved in drafting REACH Implementation Project RIP 3.3.2
Technical Guidance Documents for industry

REACH and in vitro – Article 1

Aim and Scope

The purpose of this Regulation is to ensure a high level of protection of human health and the environment, including the promotion of alternative methods for assessment of hazards of substances, as well as the free circulation of substances on the internal market while enhancing competitiveness and innovation.

REACH and in vitro – Article 25

Objectives and General Rules

In order to avoid animal testing, testing on vertebrate animals for the purposes of this Regulation shall be undertaken only as a last resort.

It is also necessary to take measures limiting duplication of other tests.

THE REASON FOR ECVAM: *DIRECTIVE 86/609/EEC*

Article 7.2:

An experiment shall not be performed if another scientifically satisfactory method of obtaining the result sought, not entailing the use of an animal, is reasonably and practicably available.

Article 23:

The Commission and Member States should encourage research into the development and validation of alternative techniques which could provide the same level of information as that obtained in experiments using animals, but which involve fewer animals or which entail less painful procedures, and shall take such other steps as they consider appropriate to encourage research in this field.

What are “Alternative Methods”?

Reduce



Less animals/group
Better experimental design

Refine



Less painful procedures
Humane endpoints

Replace



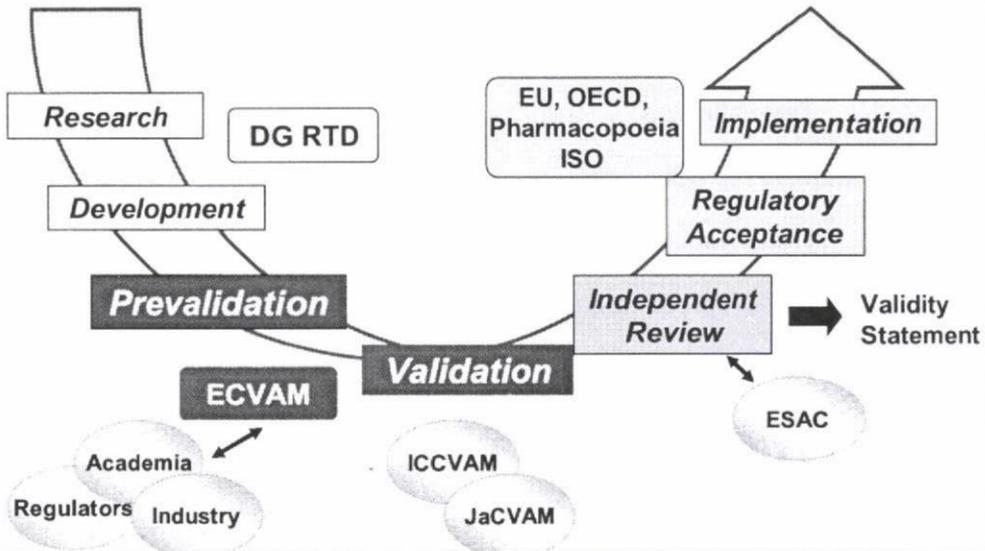
When is formal validation necessary?

... formal validation is necessary when the introduction of new tests would alter existing legislation.

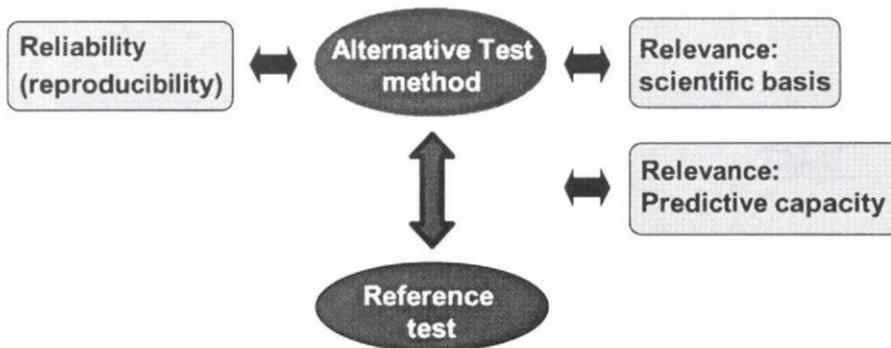
- EU directives
- OECD guidelines
- European Pharmacopoeia monographs
- ICH

Regulate the safety testing of chemicals,
cosmetics, pharmaceuticals, biologicals etc

Stages in the evolution of regulatory tests



Validation of alternative methods



VALIDATION

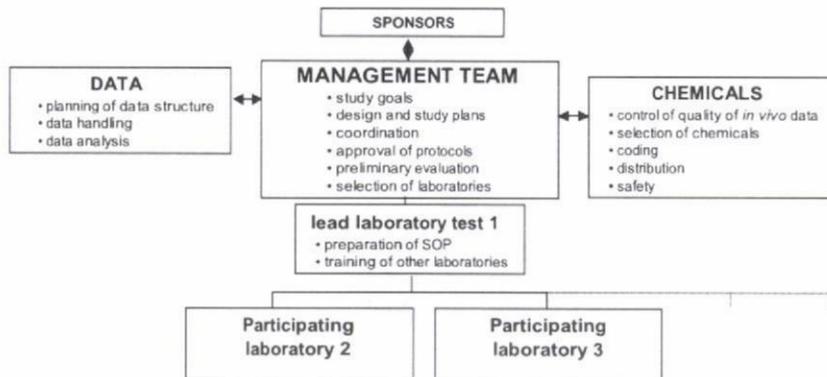
Independent assessment of the scientific basis and reproducibility of a test system, and the predictive capacity of an associated prediction model

The Modular Validation Approach

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

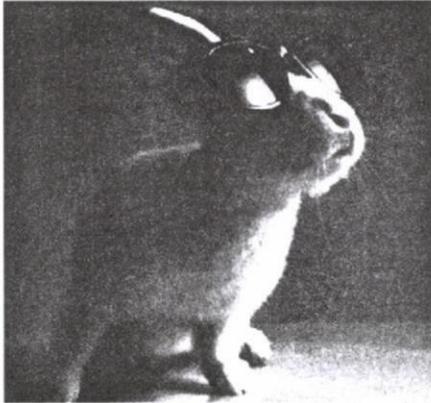


MANAGEMENT AND ORGANISATION OF VALIDATION STUDIES



Reference: Balls et al. (1995), ATLA 23, 129-147.

3R Success stories



OECD acceptance of validated methods

- 1999-2001
 - *Refine*: Painless test for skin sensitisation
 - *Reduce*: Animal numbers for acute oral toxicity from 45 to 8 (REACH: 1.1 million saved)
- 2004
 - Replace*:
 - phototoxicity
 - skin corrosion

Progress ECVAM activities in 2007

	Development	Prevalidation	Validation	Regulatory acceptance	Follow-up regulatory acceptance
<i>Skin Corrosion</i>	✓	✓	✓	✓	ongoing
<i>Acute Phototoxicity</i>	✓	✓	✓	✓	
<i>Skin Absorption / Penetration</i>	✓	✓	✓	✓	
<i>Skin Irritation</i>	✓	✓	✓	ongoing	
<i>Eye Irritation</i>	✓	✓	✓*	ongoing*	
<i>Acute Toxicity</i>	✓	✓	ongoing*		ongoing*
<i>Genotoxicity / Mutagenicity</i>	✓	✓	✓*	✓*	ongoing*
<i>Skin Sensitisation</i>	✓	ongoing	✓*	ongoing*	ongoing*
<i>Reproductive & Developmental</i>	✓	✓	✓*	ongoing*	
<i>Toxicokinetics / Metabolism</i>	✓	✓			
<i>Carcinogenicity</i>	✓	ongoing*			
<i>Subacute & Subchronic Toxicity</i>	✓				

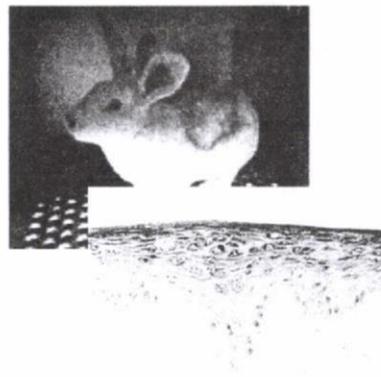
* Reduction / refinement alternatives

ECVAM's Key Areas

- Systemic toxicity
- Topical toxicity
- Sensitisation
- Carcinogenicity
- Reproductive toxicity
- Toxicokinetics
- Ecotoxicology
- Biologicals
- SIS database
- Strategic developments
(GLP, GCCP, HTS, toxicogenomics)
- Working groups composed of members of the ECVAM staff and external experts have been appointed for all the key areas

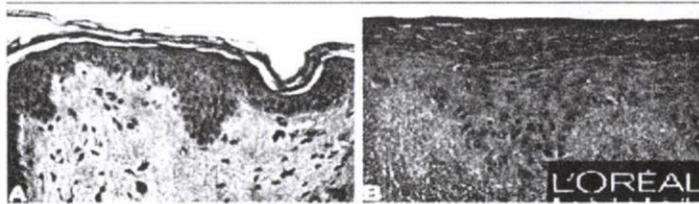
Validated 2007: Skin irritation

- Rabbit test for drugs, chemicals and cosmetics introduced 60 years ago
- Validation study 2003-2006 of three models with 9 labs (2 U.S.), 58 test chemicals
- Best model (EPISKIN), optimized in an FP4 DG RTD contract, represents a full replacement



Artificial human skin

- Biotechnology product originally to treat burn patients
- 5 European and 1 American producer
- EPISKIN opens the avenue for others to follow
- 2009 deadline of cosmetics directive, 10.000 REACH substances

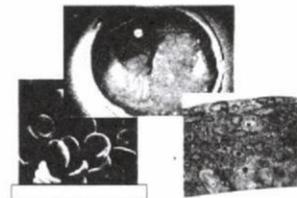


Skin

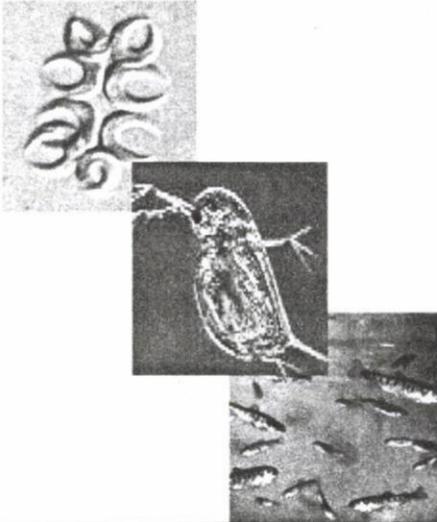
Reconstructed epidermis EPISKIN

Validated 2007: Eye irritation

- Retrospective evaluation with U.S. ICCVAM
- 4 tests analyzed, 2 qualify for the detection of severe eye irritants confirming an ECVAM analysis of 2003
- 8 other assays and the suitability for mild irritants currently under evaluation
- Intense collaboration with COLIPA
- REACH 10.000 substances

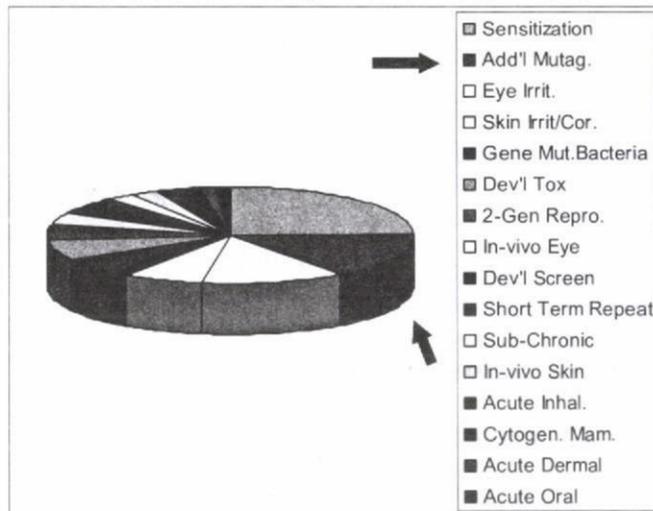


Validated 2006: Acute Ecotoxicology

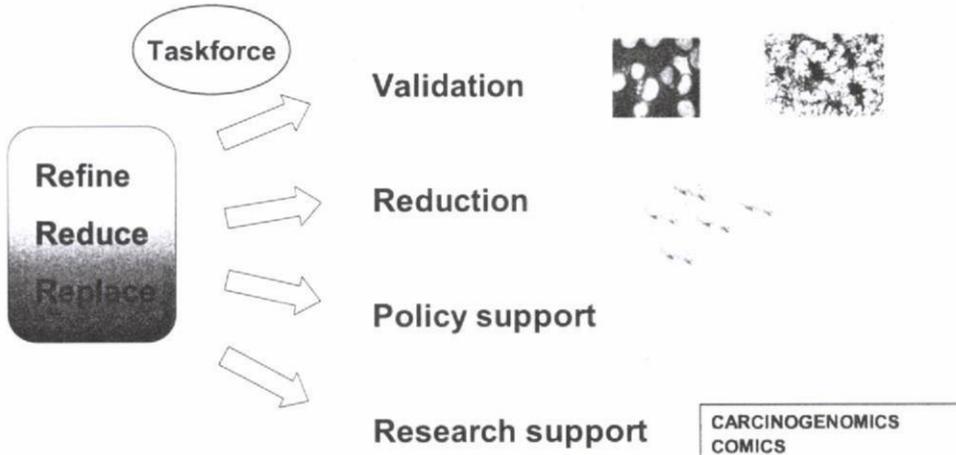


- Test strategy developed and validated by ECVAM and European Chemical Bureau within two years
- Saves 60% of fish
- Submitted to OECD, REACH

Estimated testing requirements for toxicity: % of total # substances, REACH standard scenario



ECVAM key area genotoxicity / carcinogenicity



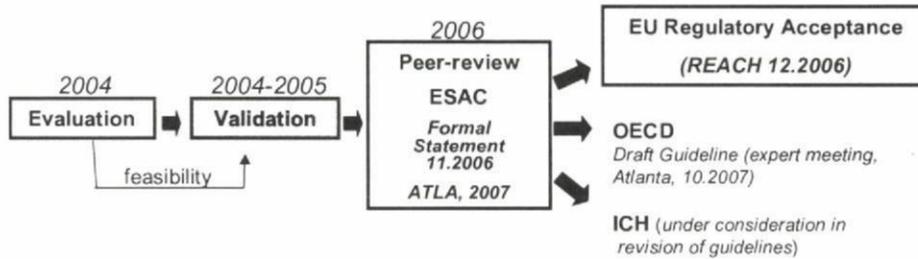
Validated 2006: Micronucleus Test *in vitro*

- Micronucleus test improves *in vitro* mutagenicity / genotoxicity assessment
- First validation based only on a compilation of existing data, no new study
- Completed in two years, within one month included in REACH legislation
- Currently considered by OECD and by ICH
- Accelerated validation



Conclusions

The *in vitro* MNT is a scientifically valid alternative to the *in vitro* CAT for genotoxicity testing



Cell Transformation Assay (CTA)

- Detects genotoxic and non-genotoxic compounds
- Mimics tumor formation *in vitro*
- Not formally validated
- Prevalidation focused on reproducibility



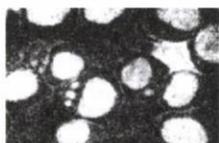
Considered at the OECD: Detailed Review Paper DRP31 on CTAs available

CTA validation
First ECVAM study involving Japan, US, EU

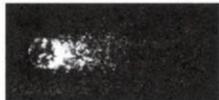


**Genotoxicity tests in 3D skin models
collaboration with COLIPA**

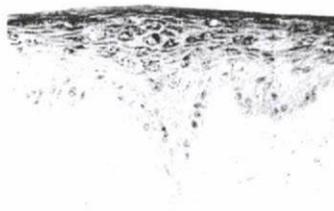
Micronucleus test



Comet assay



3D-reconstituted skin model



Reduction possibilities in genotoxicity tests *in vivo*



- Use only 1 sex
- Combine different assays in the same animal (eg. 28 days sub-chronic toxicity and micronucleus)
- Different time points in same animal
- Omit positive and/or negative controls

Coaching development of alternatives (EC projects, 30 groups/project)



Development of a novel approach in hazard and risk assessment of reproductive toxicity

Optimisation and prevalidation of an *in vitro* test strategy for predicting human acute toxicity



Novel Testing Strategies for In Vitro Assessment of Allergens

High throughput genomics-based test for assessing genotoxic and carcinogenic properties

