



# ECVAM progress report to SACATM 12'05

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<http://ecvam.jrc.it>





## Coaching and steering of DG RTD projects

- IP **ReProTect** started 7'04
- IP **A-Cute-Tox** started 1'05
- IP **Sens-it-iv** start 10'05
- STREP **Predictomics** started 9'04
- STREP **ToxDrops** started 1'05
- STREP **Vitrocellomics** start 11'05
- Marie-Curie **PulmoNet** started 4'05

**Impact: Total funding > 40 million Euro, > 120 partner institutions**

## Own research

- **Link to other units (ECB, PCE, BMS, GMO)**
- **Ecotoxicology test strategy (with ECB) under peer-review (potential of saving 60% of fish for acute ecotox.)**
- **Cancer assay now under validation**
- **8 Ph.D. (Adler, Boveri, Dodevich, Gartlon, Hoffmann Kinsner, Pellizzer, Malerba)**
- **Original publications including Nature and PNAS, reports/reviews/citations in Nature, Science, Scientific American (1'06)**





# The new dimension of development of alternative methods

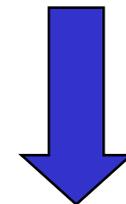
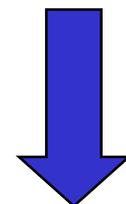
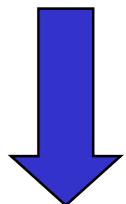
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**DG RTD**

**DG JRC  
ECVAM**

**Policy-DGs  
ECB (DG JRC)**



**Collaboration in 3 Integrated Projects**

(about 90 partners & 30 million Euro)

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





## Optimisation of validation process

- **New modular approach** → OECD doc. 34
- **Report on validation of toxicogenomics**
- **2 workshops (one internal and one on invalidation)**

## Ongoing “late-stage” validation studies

- **7 tests:** skin irritation (3), acute toxicity (2), cancer (2)
- **10 retrospective validations:** eye irritation (8), mutagenicity (1), sensitization (1)
- **9 peer-reviews:** myelotoxicity (1), pyrogen (5), chronic toxicity (1), reprotox (1), mutagenicity (1)
- **15 prepared:** pyrogen, skin irritation, photopotency, sensitisation (4), ecotox, genotox, Limulus, reprotox, endocrine disruption (6), vaccine, shellfish toxins
- **1 validated:** acute fish toxicity

## Workshops

- **5 workshops:** eye irritation (ICCVAM), reproductive toxicity, neurodevelopmental toxicity, vaccines, validation (2)
- **6 reports:** sensitisation, QSAR, Good Cell Culture Practice, toxicogenomics, fertility, immunotox.



# *Europe goes alternative*

## **Conference, Brussels, 7<sup>th</sup> of November 2005**

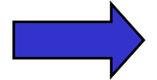


- **Hosted by Commissioners G. Verheugen (DG ENTR) and J. Potocnik (DG JRC / DG RTD)**
- **Opinion leaders from politics, science and industry**
- **300 participants**
- **A portfolio of activities in Europe**
- **European Partnership**





# *5<sup>th</sup> World Congress in Berlin: 980 participants*



**ECVAM well represented:**

- **19 Talks, 19 Posters**
- **5 Chairmanships of sessions**
- **Booth**
- **Plenary Talk**
- **GCCP document for all participants**
- **Who-is-who flyer**
- **CD with first 50 workshop reports**
- **ECVAM and EFSA responsible for WC7 in 2009**



# *GCCP published*



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

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**GLP**

**ECVAM Workshop Report**      **OLIVE (ECVAM laboratories)**

**OECD Draft Consensus Document**

**OECD Advisory Document No 14**



**GCCP**

**World Conference Workshop**

**ECVAM Task Force Report**

**ECVAM draft Guidance Document**

**ECVAM final Guidance Document**

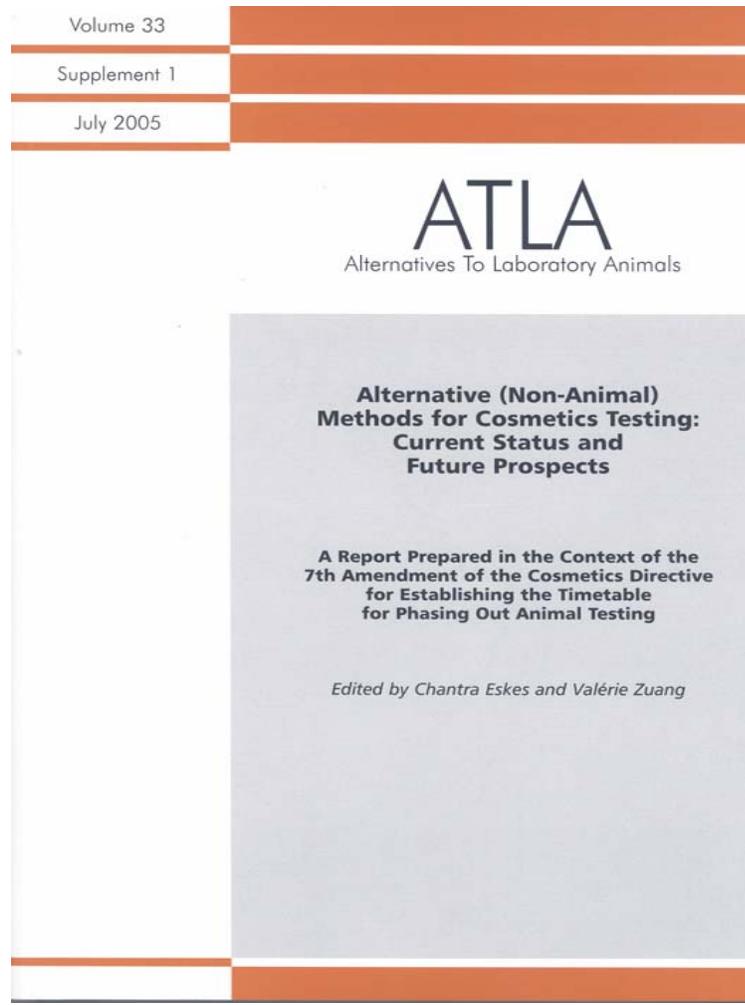
**Coecke et al (2005)**  
**Guidance on Good Cell Culture Practice;**  
**ATLA, 33:261-287**





# Status of alternative methods for cosmetics published

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**Taskforce chaired by ECVAM:**

- 5 DG of Commission
- 5 Trade associations
- 3 Animal welfare NGOs
- OECD

75 experts produced 280 page report





# *Can the same tests be applied for new and existing substances?*

## **New substances**

- **No knowledge about toxicity**
- **Low established commercial value**
- **Problem of false-negatives**

## **Existing substances**

- **Experience from use**
- **High commercial value**
- **Problem of false-positives**



# *Why Testing Strategies instead of tests?*

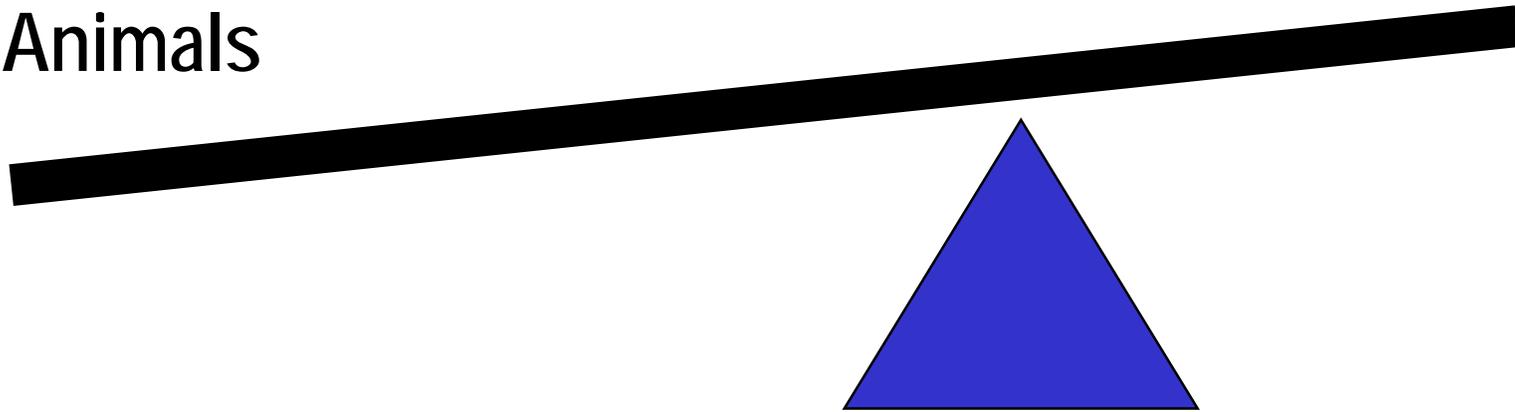
- **Single tests can be optimised only to reduce either**  
**false-positive (economical problem)**  
**or**  
**false-negative (safety problem)**  
**results**
- **Optimise work, costs, animal numbers and safety**



# *ITS purpose: finding the right balance*

Costs  
Animals

Safety





# *Components of Intelligent Testing Strategies*

- **Use of existing data**
- ***In-vitro* tests**
- **Optimised *in-vivo* tests**
- **Thresholds of toxicological concern**
- **SARs / QSARs and modelling**
- **Read-across and chemical categories**
- **Exposure assessment/exposure-based waiving**



## *Towards ITS in RIP 3.3*

- Support to ECB
- Steering Group with CEFIC / ECETOC / others
- Continuation of Project Management Group and Stakeholder Expert Groups with MS
- Expert Nomination Process
- Continuous transparency to DGs, ECB and stakeholders
- „Think tank“ ITS

### ECVAM taskforce

- T. Hartung
- J. Riego Sintes, ECB
- L. Scott, P&G, seconded to ECVAM
- M. Kayser, BASF
- Patric Amcoff, SAWA and OECD, partially seconded to ECVAM



# Progress reports from Key Areas / Actions



# *Skin Irritation Validation Study*

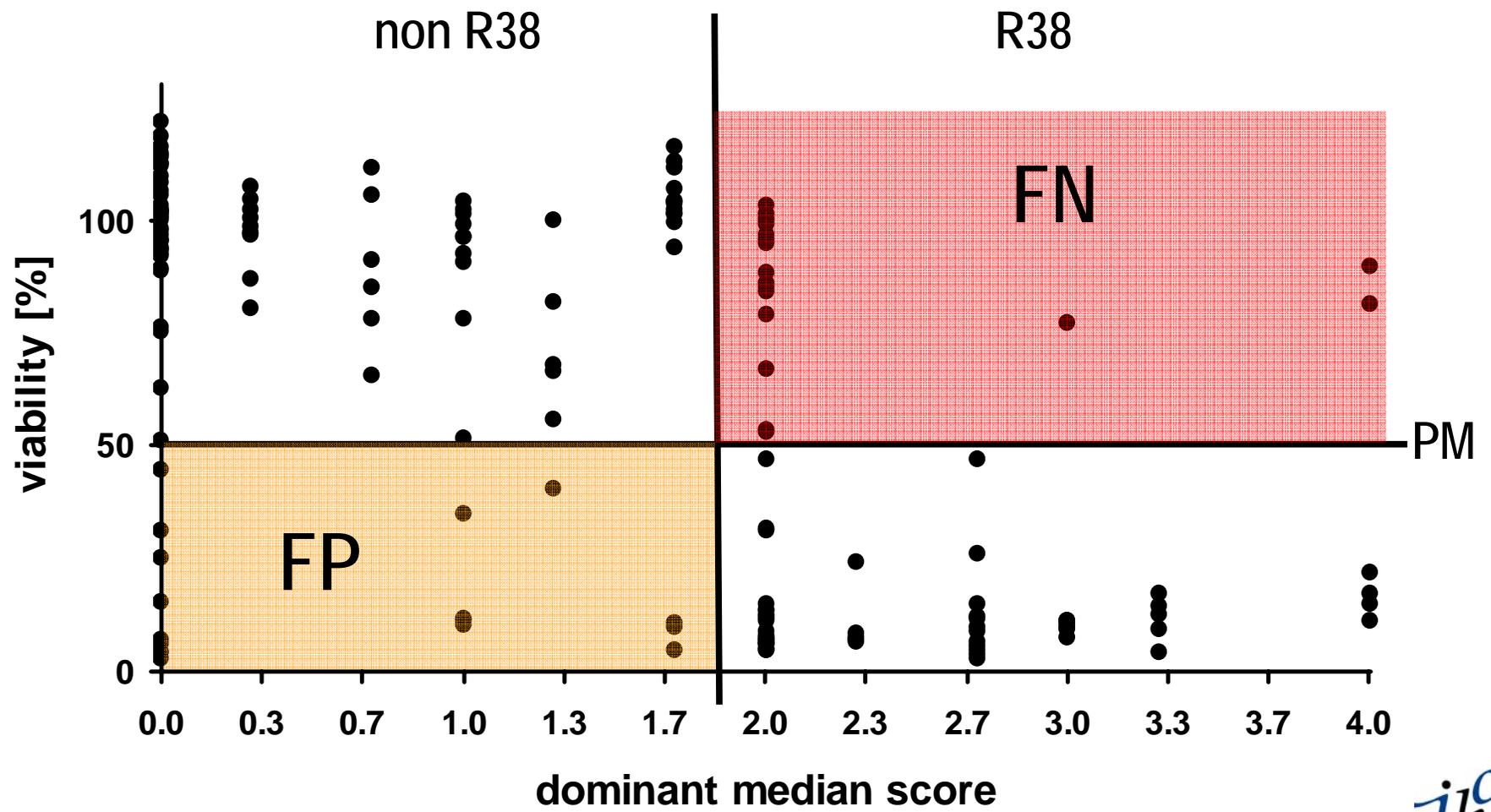
**4th MANAGEMENT TEAM MEETING**  
**ECVAM, 12-13 July 2005**

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

# Skin Irritation Validation study: Results EPISKIN

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# *Skin Irritation Validation study: Results EPISKIN*

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Predictive capacity (three laboratories)

Specificity = 82.7%

Sensitivity = 75.3%

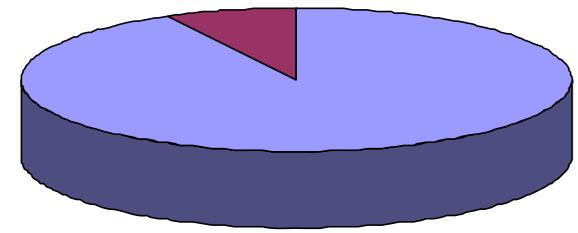
Prevalence in the NCD: 7.9%

➔ NPV = 97.5%

PPV = 27.2%

➔ Identify the negatives

Prevalence of skin irritants  
in the New Chemicals Database  
(n = 2327)





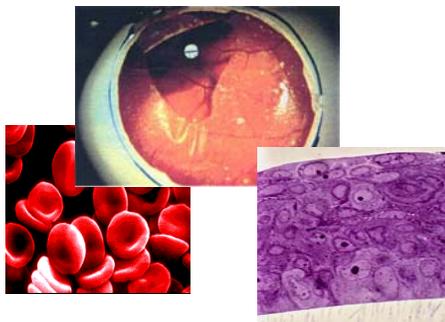
# Eye Irritation – Collaboration EU & US

## Evaluation of methods (with ICCVAM)

*Organotypic methods*

*Reconstituted human tissue models*

*Cell cytotoxicity- / function- based methods*



## Three joint workshops in 2005

## Review of the in vivo Draize Eye Test

*Based on literature and evaluation of existing data*

## Evaluation of LVET





# Key Area Sensitisation

- **Dendritic Cells as a Tool for the Predictive Identification of Skin Sensitisation Hazard**  
The report and recommendations of ECVAM Workshop 51, ATLA 33, 2005
- **May 2005 Formal approval of Sens-it-iv**
- **Taskforce meeting on skin and respiratory sensitisation**  
Ispra, 20-21 September 2005
- **Evaluation of non- radioactive LLNA methods**
- **Evaluation of the LLNA limit test**
- **Analysis of skin sensitisation prevalence in the NCD**
- **Ad hoc expert meeting on percutaneous absorption planned**
- **Workshop on chemical respiratory sensitisation planned**
- **Validation activities planned for 2006 (3 tests, one strategy)**



**Management team**  
 Bill Stokes (ICCVAM/US)  
 Patricia Pazos/Miriam Jacobs (ECVAM/EU)  
 Alberto Mantovani (ISS/IT)  
 Craig McArdle (Univ. of Bristol, UK)

**Chemical Selection**  
 Karin Aschberger (ECB)

**Protein binding assays**

**ER binding assay (Pan Vera)**  
 1. lab: Research Triangle Park/US EPA)  
 2. lab: Bayer/ Germany  
 3. lab: CER/Japan

**AR binding assay (Pan Vera)**  
 1. lab: Bayer/ Germany  
 2. lab: Research Triangle Park/US EPA  
 3. lab: Institute of Applied Microbiology /A

**Transcriptional activation assays**

**AR-Calux (USO2 cells)**  
 1. Bio Detection System  
 2. laboratory: Vito/ Belgium  
 3. laboratory: Bayer

**PALM (PC3 cells)**  
 1. lab: Bayer  
 2. lab: Vito/Belgium  
 3. lab: NN

**ER-Calux (T47 D cells)**  
 1. laboratory: Bio Detection System  
 2. laboratory: Vito/ Belgium  
 3. laboratory: Bayer

**MELN (MCF7 cells)**  
 1. lab: Vito/Belgium  
 2. lab: Bayer  
 3. lab: NN

4/11

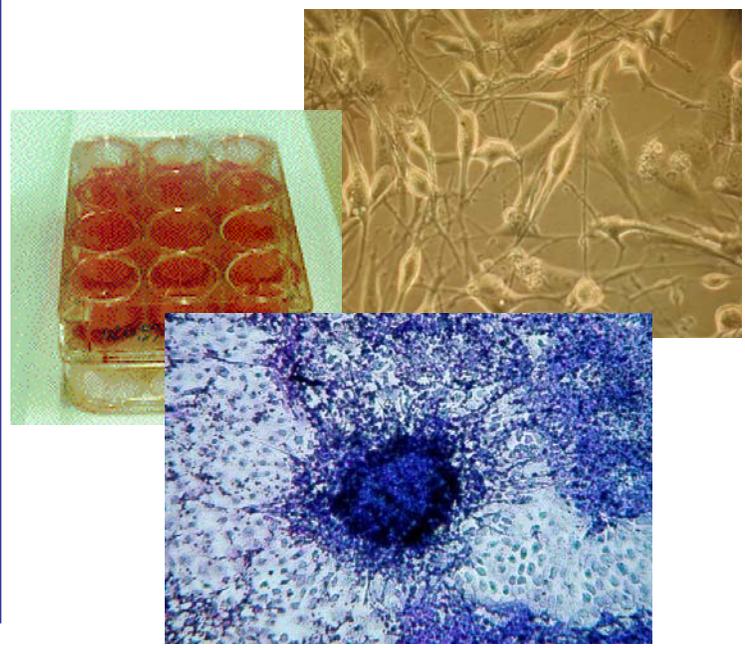


# 1st study involving EU, USA & Japan

## Carcinogenicity

The only regulatory accepted test:  
2 years animal test (1 M€/chemical)

Alternative: *in vitro* Cell Transformation Assay



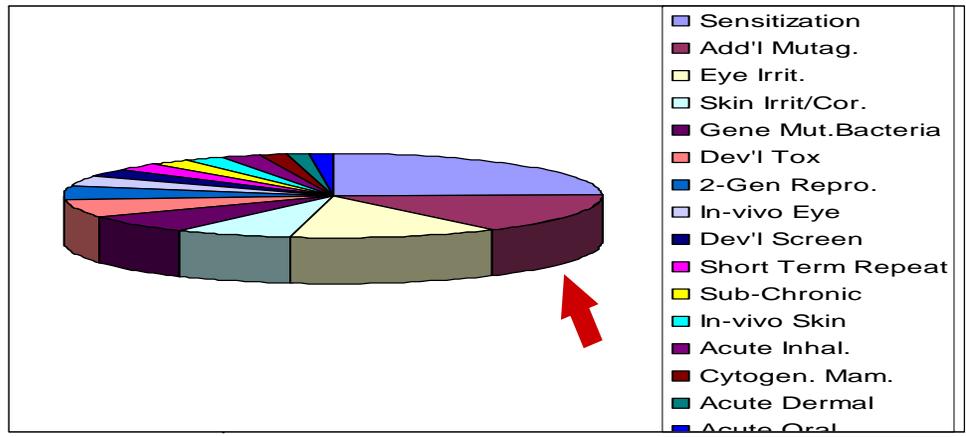
Prevalidation initiated in 11/2004

# Mutagenicity/Genotoxicity

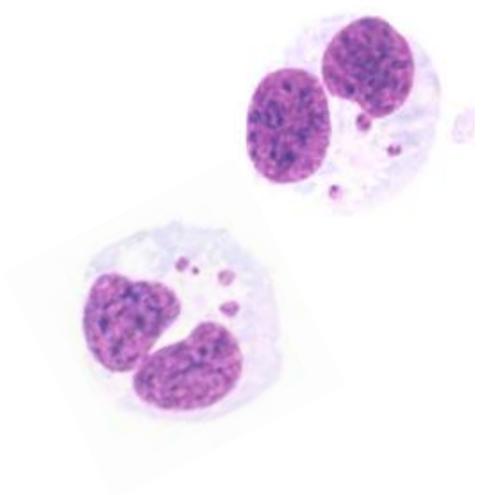


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2<sup>nd</sup> largest testing requirement  
**REACH**



## *Micronucleus Test in vitro*



- 1<sup>st</sup> retrospective validation
- Peer review starting
- Parallel OECD activity



# Biologicals



- Meeting with EDQM and WHO March 2005:  
Identify methods to replace intracerebral challenge in Kendrick test (potency testing of whole-cell pertussis vaccines)
  - ➔ Start of 2 ECVAM studies to optimise serological tests
- Start of pyrogenicity peer review process end of June 2005
- ECVAM/DG SANCO workshop on 3Rs Approaches in Marine Biotxin Testing – draft report in consultation with experts
- Scientific advice to CRL (Vigo) on prevalidation of functional assay for PSP testing
- Botulinum toxin: ECVAM in contact with EDQM regarding meeting with manufacturers, no date fixed
- Comments on Ph.Eur. Monographs published in Pharmeuropa



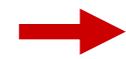
# An Intelligent Testing: Ecotoxicology

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**Today**  
Concentration killing 50% of algae, water flea and fish is determined (48 to 60 fish) → lowest value

## Future



**60% less fish use**

Algae and water flea tested first  
only lower value then tested in 10 fish  
only 16% of substances require further fish tests





- **Threshold/Step-down Approach**
  - *Jeram et al., RTP 42: 218-224*
  - Evaluation of ecotoxicological data of existing chemicals of IUCLID Database (ECB), plant protection products, pharmaceuticals with threshold approach
  - ESAC Peer Review Threshold/Step-down approach:
    - compilation of documents
    - establishment of panel
    - Considered valid 9'05
  - Presentation of Threshold/Step-down approach at 7<sup>th</sup> Meeting of Technical Committee of New and Existing Chemicals of ECB – 14/09/2005 (*Juan Riego might report on outcome*)
- **Task Force meeting in July dedicated to "bioconcentration"**
- **Meeting at UBA on Fish Embryo Test (September 2005)**
  - Submission of data for ESAC Peer Review planned for end of 2005
- **Launch of study on optimisation of cytotoxicity tests using fish cells**



# Toxicokinetics



- **Physiologically-based pharmacokinetic modelling (PBPK)**
  - Preparatory expert meeting held in May 2005
  - Workshop to be held October 10 – 12.
- **In vitro kinetics**
  - fate of compounds in vitro, in vitro exposure
  - Workshop in preparation (early 2006)
- **Work on Barrier models**
  - New 18-month prevalidation study of GI absorption in vitro models will start.
  - One-year study to evaluate 5 in vitro BBB models is planned
- **Use of human hepatocyte culture**
  - prevalidation study of the predictive capacity for induction (3 labs)
  - Study finalised, Report in preparation
- **ECVAM in-house in vitro kinetics research**



# Strategic Developments



## *GLP and GCCP*

Coecke, S et al. (2005) Guidance on good cell culture practice. A report of the second ECVAM Task Force on good cell culture practice. *ATLA* 33 (3), 261–287 ->distributed at World Conference

## *HTS*

HTS facility ECVAM: on the 21-22 September the ECVAM robot did the first test runs for the 3T3 NRU uptake method in Bonaduz (CH) (the robot is now being transferred to ECVAM)

## *DNT*

ECVAM-CEFIC-CAAT workshop April 2005

## *Nanoparticle toxicology*

Assessment on different in vitro cell culture methods is ongoing

## *Omics and profiling methods*

Profiling methods: ECVAM Laboratory activities are ongoing to assess different profiling methods for their predictive capacity





ECVAM DATABASE SERVICE  
ON ALTERNATIVE METHODS TO  
ANIMAL EXPERIMENTATION

## Current Activities & Prospects

- Online availability of the whole DB-ALM data contents as an Oracle based database service (foreseen end 2005)
- Data collections on Reproductive Toxicity and Eye Irritation (>2 years)
- Extension of DB-ALM to include information on computational toxicology including QSARs
- Maintenance of the ECVAM website



# Common goals

