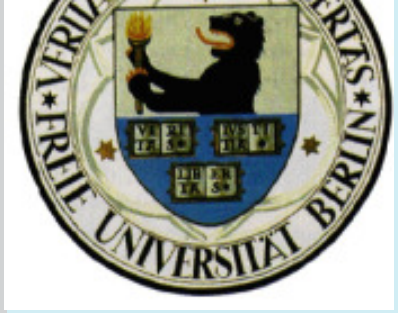



BfR
Risiken erkennen – Gesundheit schützen

FEDERAL INSTITUTE FOR RISK ASSESSMENT

# Symposium
# 20th Anniversary of ZEBET at BfR and 50 Years of the *3Rs* Principle

**October 26-27, 2009**

**Federal Institute for Risk Assessment (BfR), Berlin,**

# 20 Years of ZEBET 1989-2009: *A Success Story*

**Horst Spielmann**
**Professor for Regulatory Toxicology**
**FU Berlin und BfR, Berlin**

# Topics

- Establishing ZEBET at the BfR
- ZEBET's "Mission" and legal status
- ZEBET's Data Base and Information Service
- ZEBET's Funding of Research
- ZEBET's *"in house"* Research Activities
- ZEBET's Validation and Evaluation Activities
- ZEBET's future

# ZEBET 1989

**At the opening symposium of ZEBET in 1989 the representative of the Ministry of Health has defined ZEBET‘s task in the following way:**

***“ZEBET has primarily the task to protect experimental animals in Germany and it is the only government agency in Germany with this mission. In contrast there are many established government agencies for the protection of humans, e.g as consumers, patients and workers.“***

**Has this task changed over the past 20 years and what about the future??**

# ZEBET 2009 ➔ 2020

# EU DIRECTIVE 86/609/EEC
# on the use of experimental animals

## 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. The commission and Member States shall monitor trends in experimental methods.**

# EUROPEAN CENTRES FOR ALTERNATIVES TO ANIMAL TESTING

1981

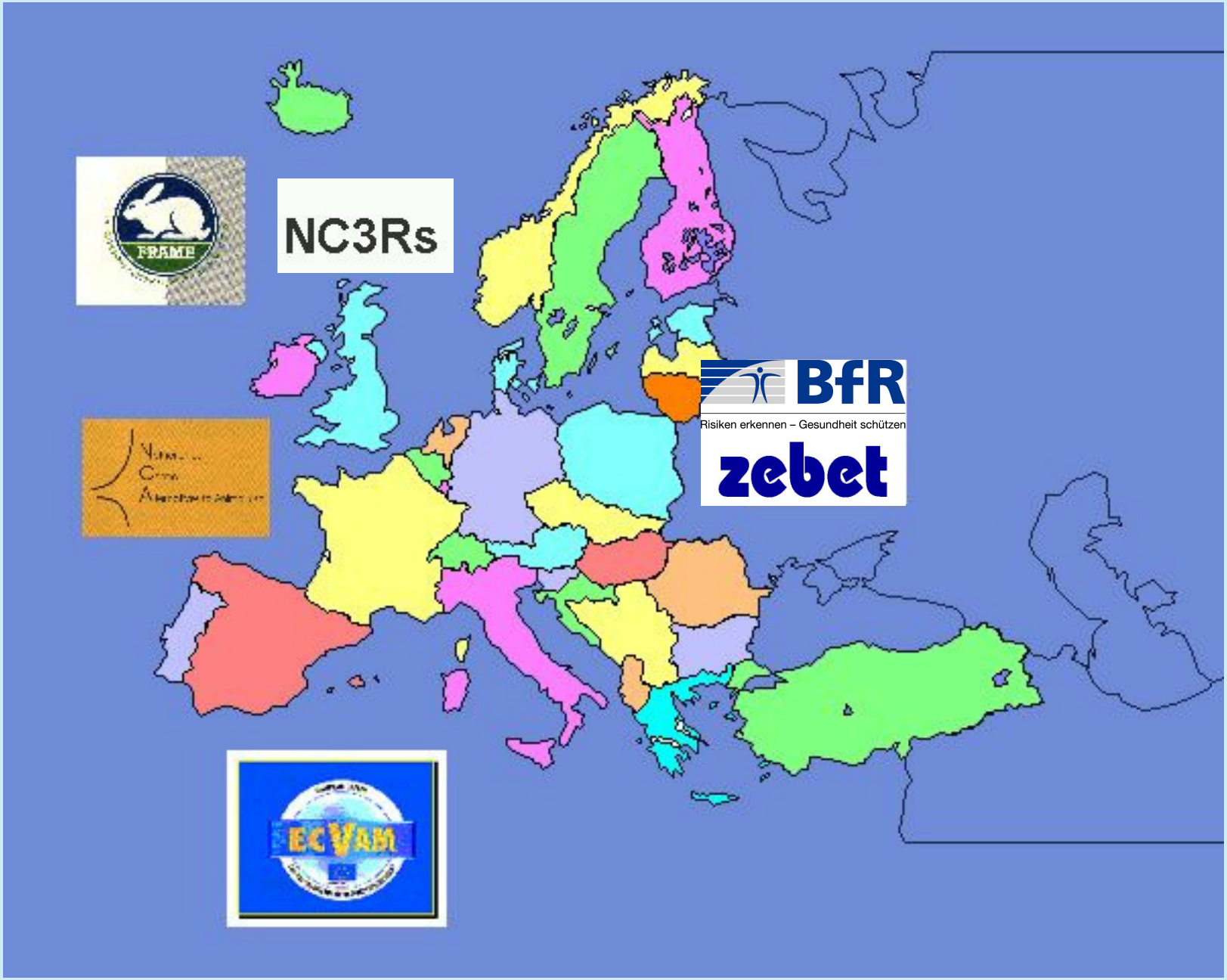
CAAT
Hopkins

1998

ICCVAM
USA

2005

JaCVAM
Japan




2001

vitryna

POLAND

**Z**entralstelle zur

**E**rfassung und

**B**ewertung von

**E**rsatz- und Ergänzungs-
methoden zum

**T**ierversuch

**N**ational German

**C**entre for

**D**ocumentation and

**E**valuation of

**A**lternatives to

**A**nimal Experiments

---


**BfR = Bundesinstitut für Risikobewertung**
**= Federal Institute for Risk Assessment**

Commodities
Biocides
Chemicals
Cosmetics
Foods
Pesticides
ZEBET
Recommendations
Databases
Research
Internationals
Publications
Masthead

Biological Safety >>>

Food Safety >>>

Safety of substances ... >>>

Product Safety >>>

## Science in the Service of Humanity

The Federal Institute for Risk Assessment (BfR) is the scientific body of the Federal Republic of Germany that prepares expert reports and opinions on questions of food safety and consumer health protection on the basis of internationally recognised scientific assessment criteria. With the help of risk analysis, BfR formulates action options for risk reduction. Against this backdrop, the Institute assumes an important task in improving consumer health protection and food safety. The assessments are to be presented in a transparent and comprehensible manner to the general public, scientists and other involved or interested circles. The assessment results will, in principle, be made publicly accessible whilst maintaining the confidentiality of protected data. **more >>>**

## Enjoyment with unpleasant consequences

The Shiitake mushroom (Lentinus edodes) is popular in Germany as an edible mushroom. It is grown and eaten in large quantities. However, in the case of particularly sensitive people this culinary enjoyment may be somewhat dampened later by striated, lash-like reddening of the skin. The trigger of this rare, in some cases severe, skin reaction is probably a natural ingredient in this kind of mushroom. **more >>>**

### More important information

▶ 2-Ethylhexanoic acid in baby food and fruit juices packed in glass containers (BfR Opinion of 2004-07-20) 58 KB

### Europeans work togethe on controlling zoonoses

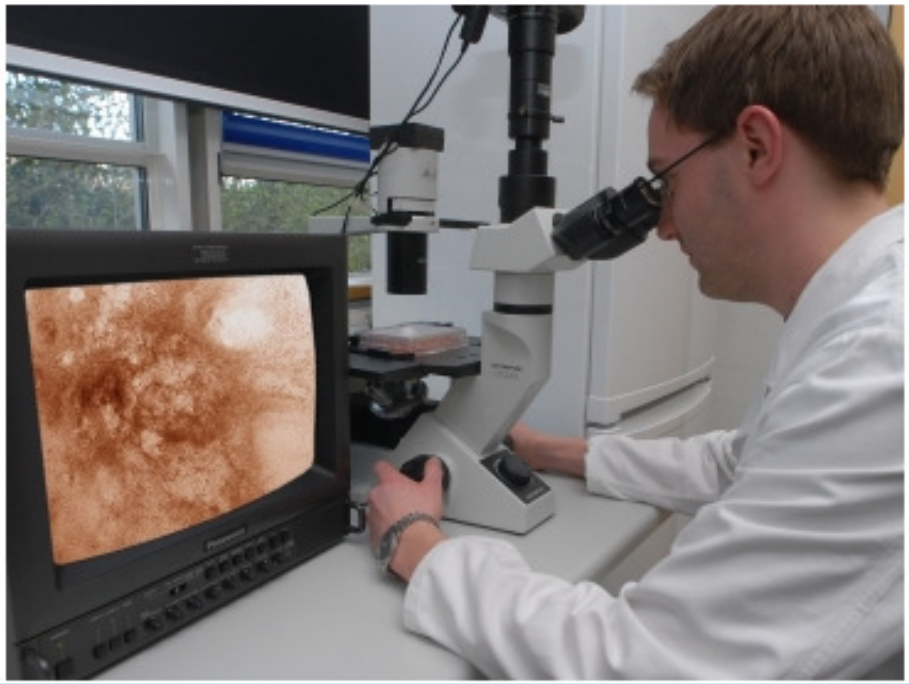
The green light has been given to the European Network of Excellence for Integrated Research on th Prevention and Control of Zoonoses, MED VET NET 300 leading scientists fron countries and 16 scientific institutions have joined for in order to develop joint strategies to control disea transmitted from animals t humans. **more >>>**

### Animal welfare and consumer protection ar reconcilable!

The new European chemic legislation leads to a noticeable improvement in health protection. BfR determines in a new study that this improvement is n necessarily linked to considerably higher numb of experimental animals a feared by various sides. Precondition is that the

# http://www.bfr.bund.de/zebet



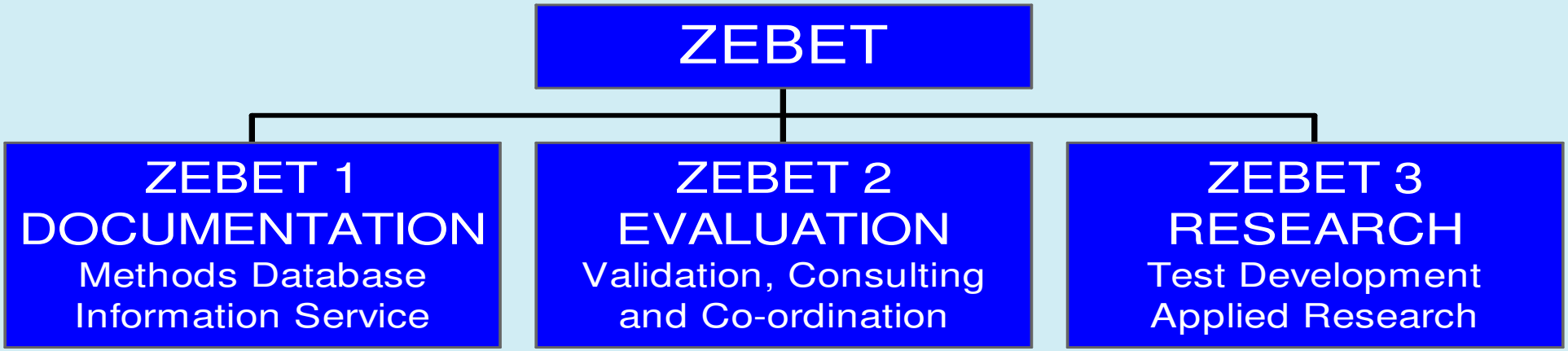



## *ZEBET - Centre for Documentation and Evaluation of Alternatives to Animal Experiments*

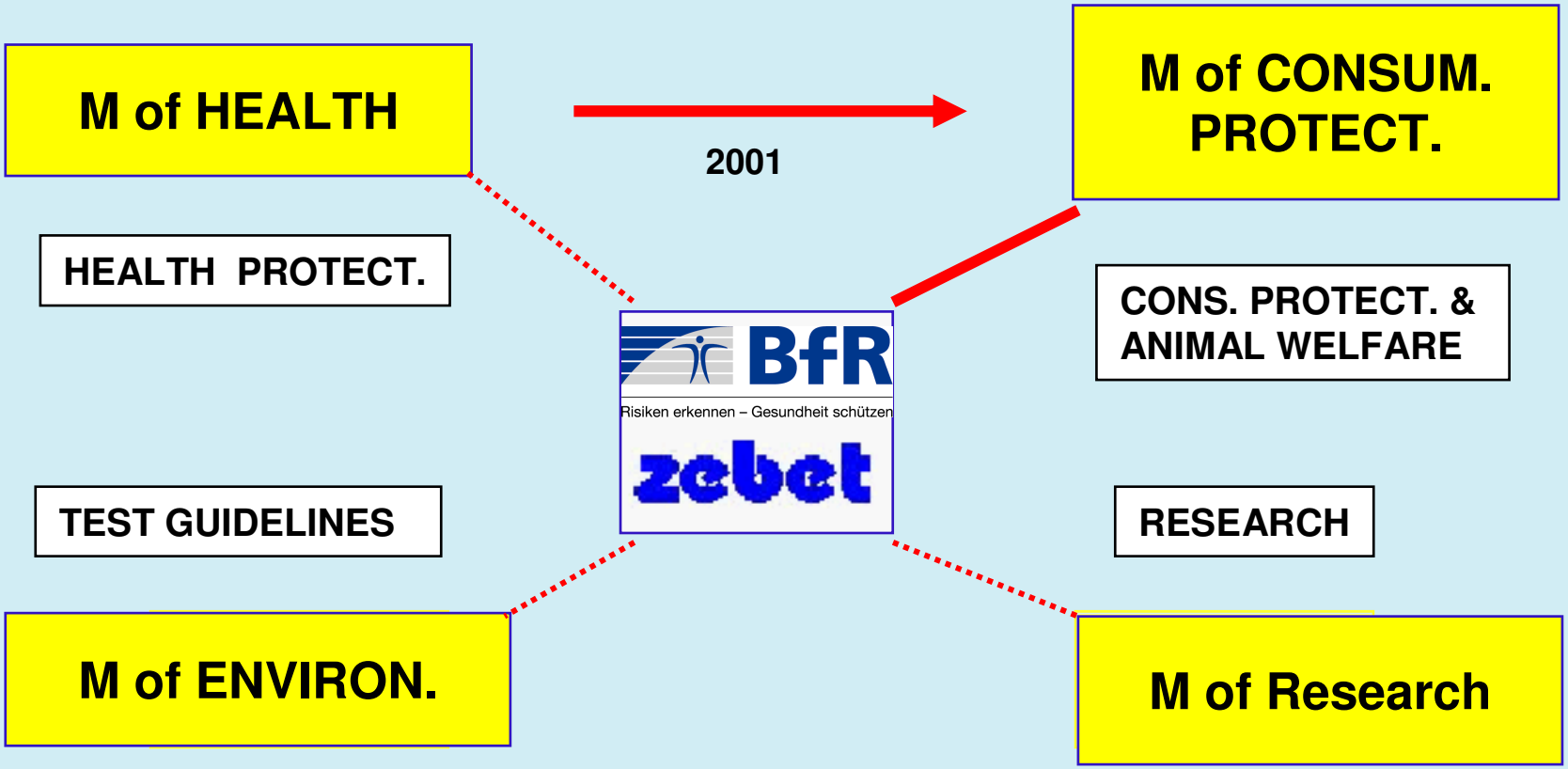
The Centre for Documentation and Evaluation of Alternatives to Animal Experiments (ZEBET) was established in 1989. For now some twenty years ZEBET has actively contributed to promote and to ensure the implementation of the 3Rs principle described by William Russel and Rex Burch in 1959 into administrative and scientific practice. To celebrate the 20th Anniversary of ZEBET and the 50th Anniversary of the publication on "The Principles of Humane Experimental Technique" by Russell and Burch, **a two-day conference will be held at BfR in Berlin on 26-27 October 2009**. The aims of this symposium are to review the contribution of ZEBET in the fields of 3Rs during the past two decades, including national and international collaborations and to discuss the

# ZEBET's Mission

- to establish a database & information service on alternatives at the national and international level
- to develop alternatives according to the "3Rs principle" of Russel and Burch
- to fund research on alternatives
- to co-ordinate validation studies
- to co-operate with national & international funding agencies and validation centres
- to provide a forum for information on alternatives to animal testing




## RELATION to MINISTRIES



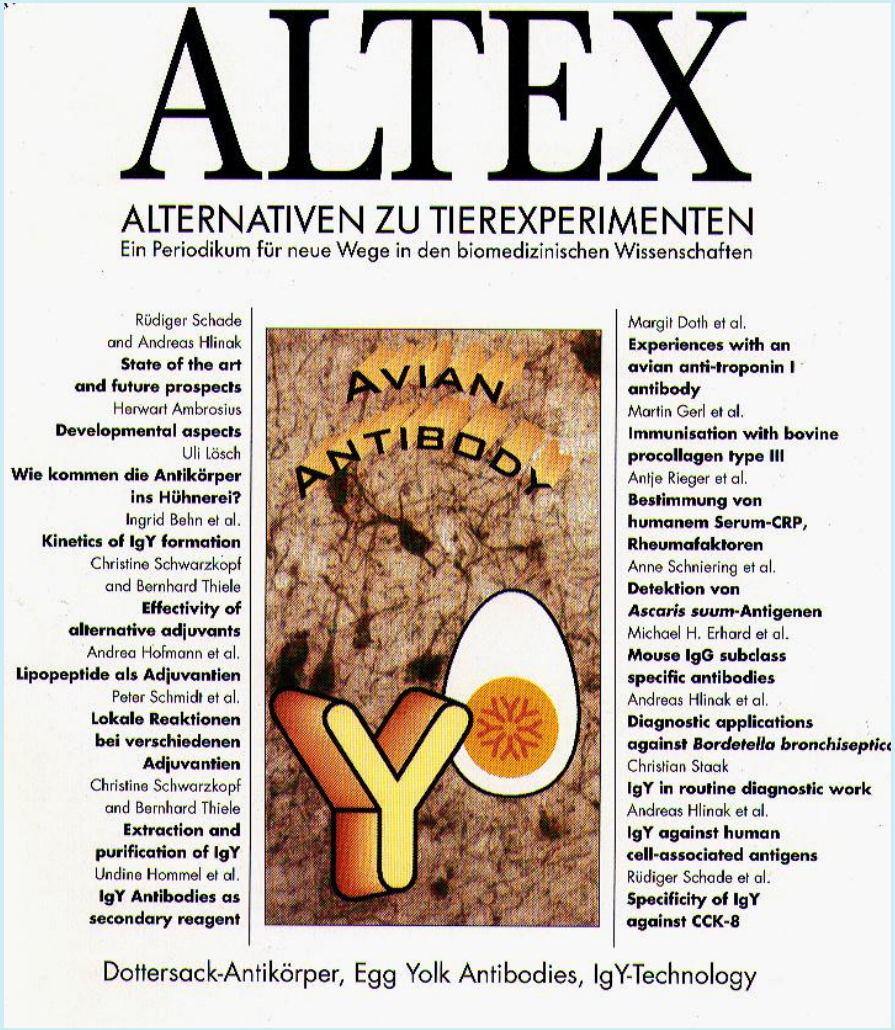
***Go3R allows to significantly reduce animal experiments by specifically identifying 3R-methods on the internet.***

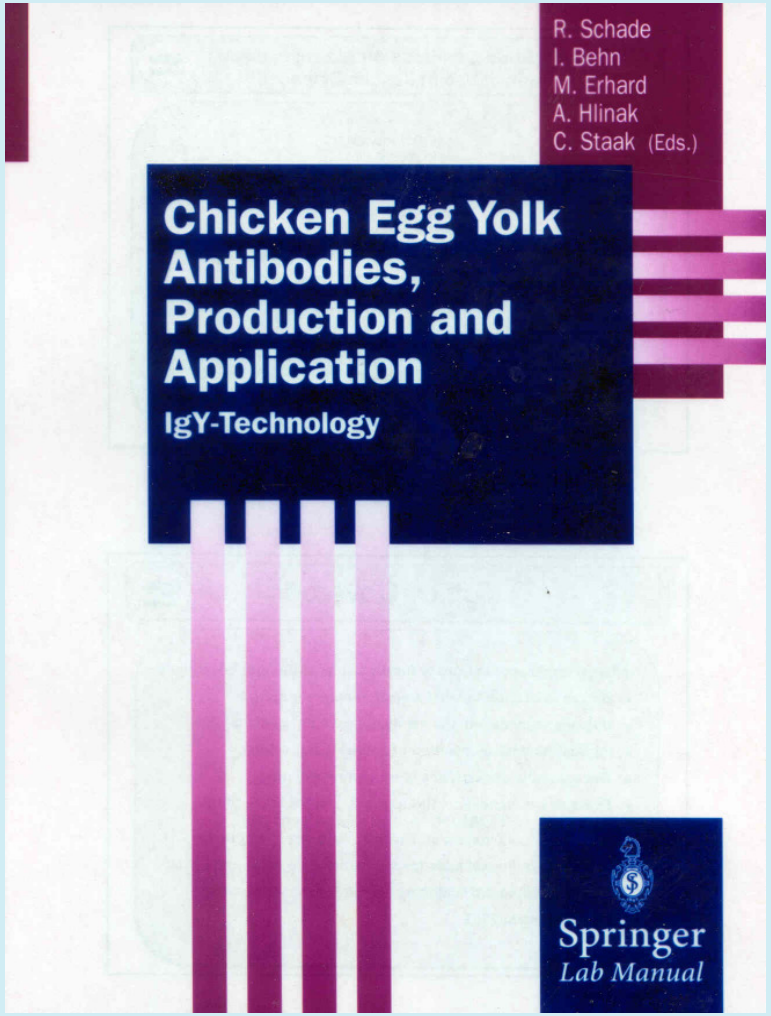
BfR

TECHNISCHE UNIVERSITÄT

Freie Universität Berlin

# Polyclonal avian antibodies: IgY technology



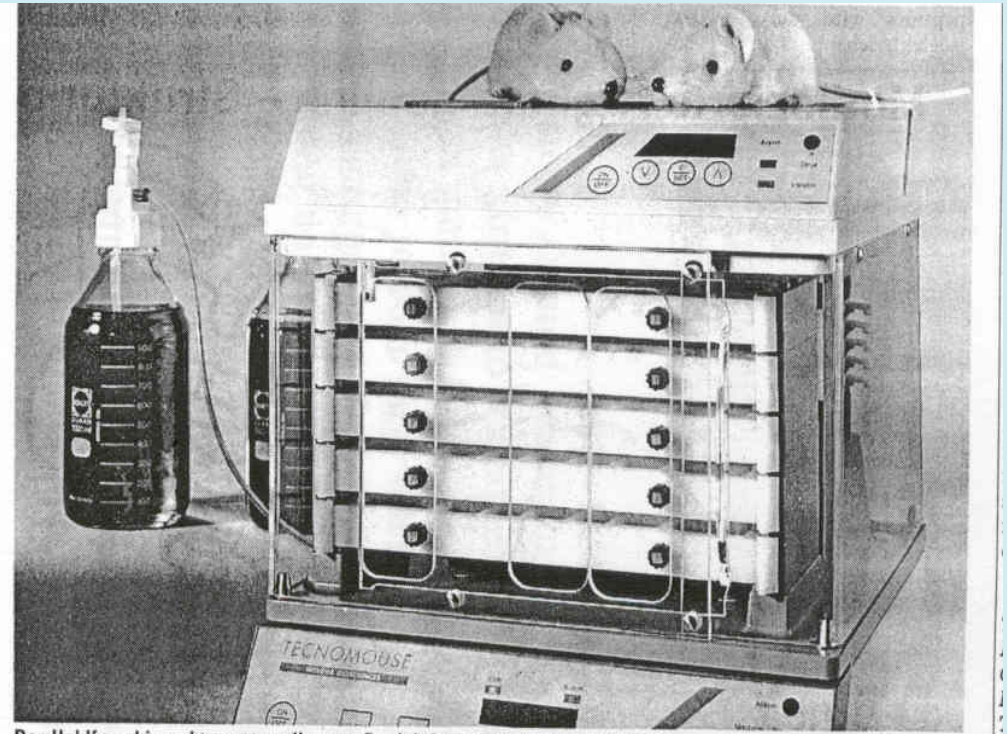




**ZEBET Workshop 1995**

**Book 1998**

# In vitro production of monoclonal antibodies: the TECHNOMOUSE

## Germany 1994




Das Hohlfaserbioreaktorsystem dient zur Produktion monoklonaler Antikörper. (Foto: zvg)

## USA 2000


## Integra Biosciences

## Calbiochem

# Pyrogenicity testing: cytokine TNF-α from human blood

## Germany 1996


INPUT
STEINBEIS-TRANSFERZENTRUM
IN-VITRO PHARMAKOLOGIE UND TOXIKOLOG

## USA 2002

endosafetimes

CHARLES RIVER
LABORATORIES

# *Cosmetics Industry and the 7th Amendment of the EU Cosmetics Directive*

**EU: 2.000 companies, 60 billion € turnover**

**EU: 5.000 new products per year, 25% turnover with products released within last 6 months**

**Marketing ban since 2003 for testing finished products in animals or not using ECVAM-validated methods**

**Phasing out testing in animals and stepwise marketing ban in 2009 and 2013**

# *The Chemical Industry and REACH*

**EU: 27.000 companies (96% SME), 590 billion € market = 33% of the world market, 1,7 million workers/employees**

**REACH: 30.000 chemicals marketed at >1 ton/year will have to be evaluated**

**86% of toxicological data are missing for existing/'old' chemicals**

**180.000 pre-registrations expected by 2009**

**70% of testing must be conducted by 2011-2017**

# Harmonised OECD Validation Concept 1996 & ECVAM, ICCVAM (USA)

**Test Development**

- basis
- need
- protocol
- prediction model

**Scheme for Prevalidation**

- optimisation protocol
- interlaboratory transferability
- optimisation prediction model

**Validation**

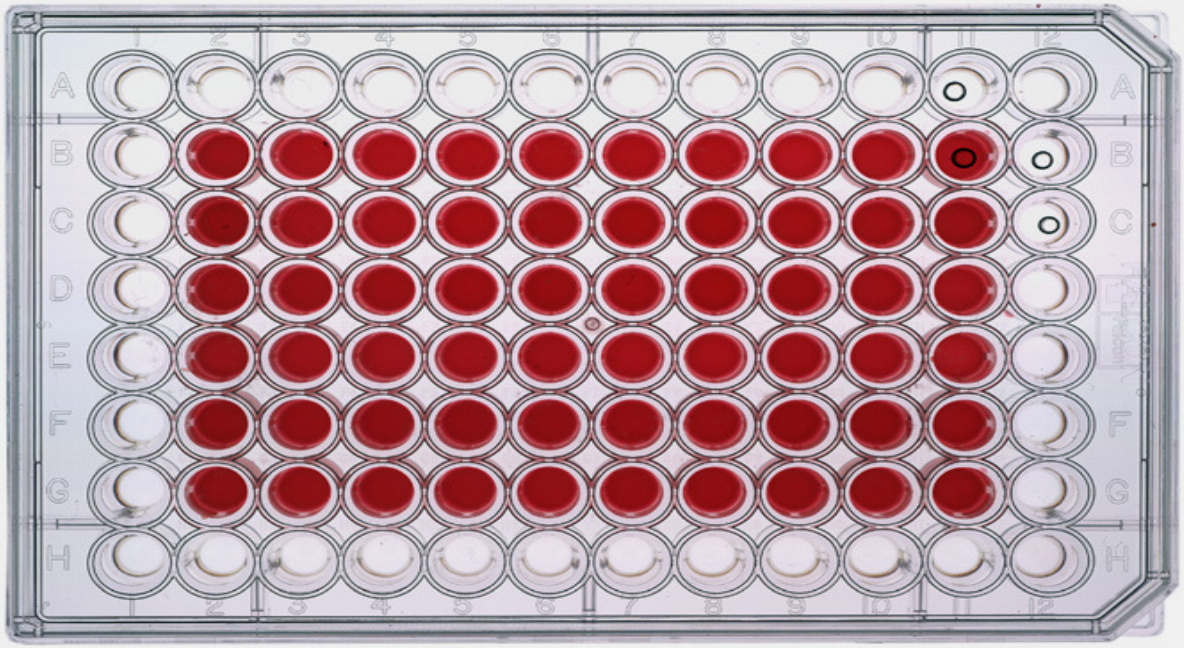
- blind trial
- relevance / reliability for a specific purpose

**Independent Evaluation**

**Regulatory Acceptance**

# In vitro phototoxicity testing

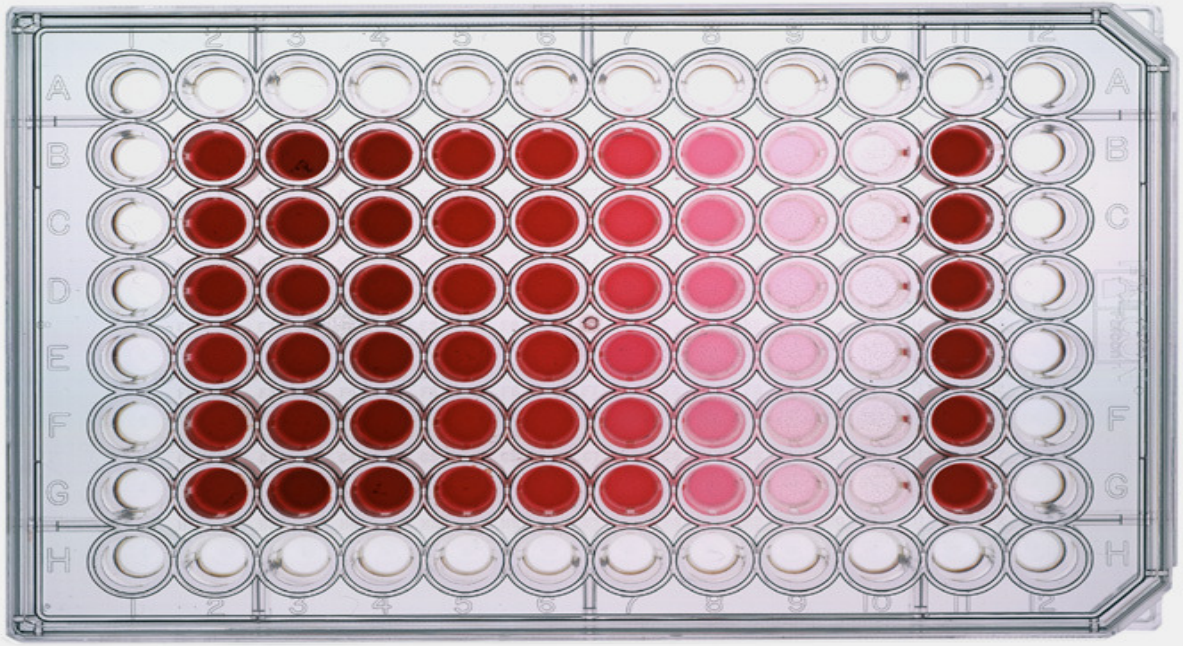
# (phototoxic but no toxicity in the dark)



Ketoprofene -UV

2 - 10 =
0 - 500 µg/ml

1 + 11 =
neg. contr.



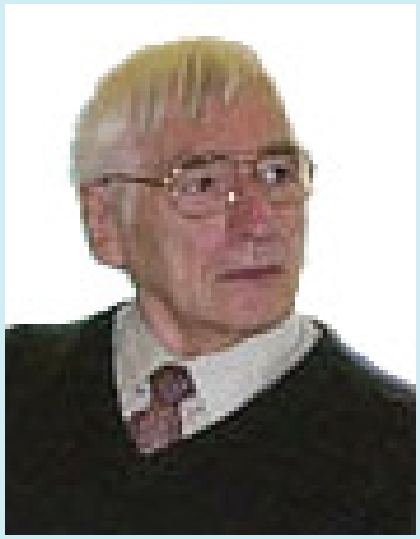
2 - 10 =
0 - 10 µg/ml

1 + 11 =
neg. contr.

# History of Cytotoxicity Testing

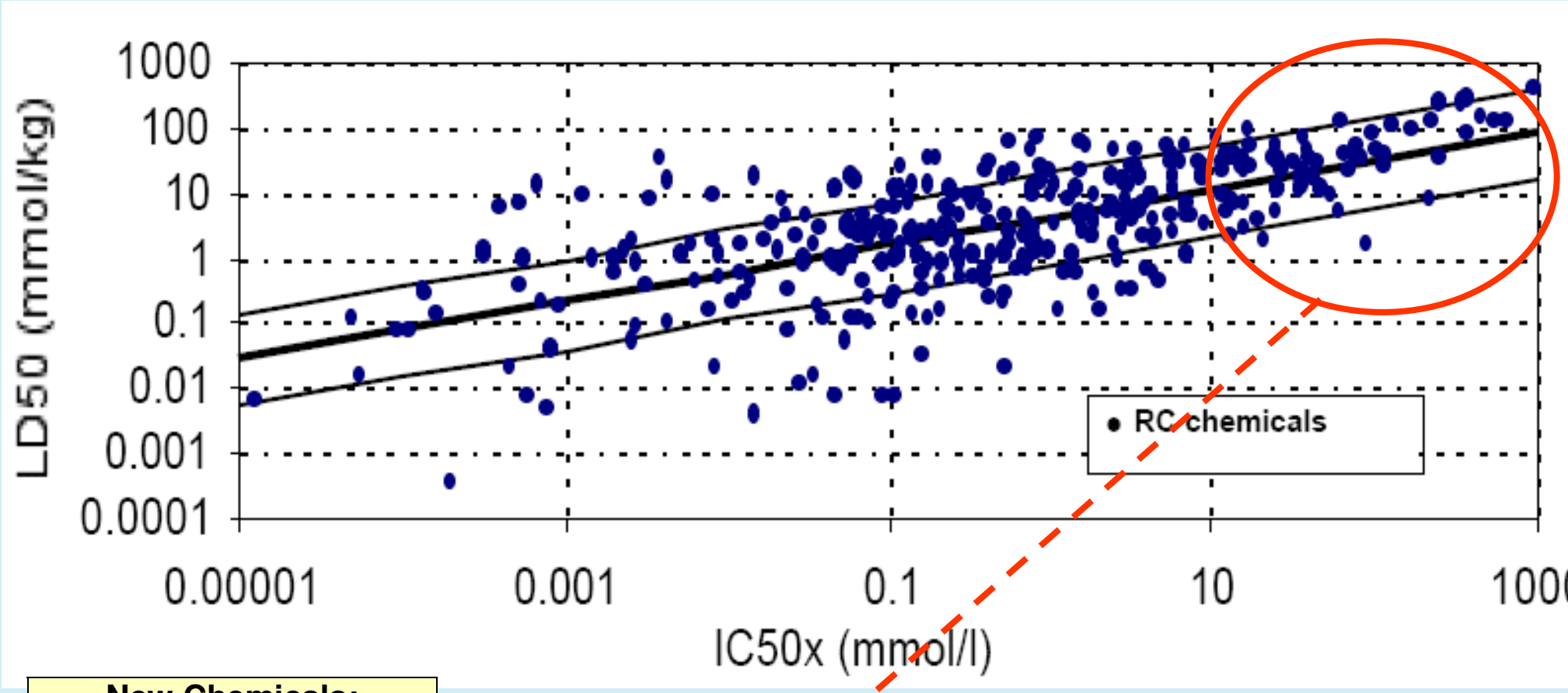
**Among many scientists proposing the concept of basal cytotoxicity for *in vitro* prediction of *in vivo* toxicity two pioneers:**

**Björn Eckwall**
**(The MEIC Programme)**



**Willi Halle**
**(The Register of Cytotoxicity)**

# Use under REACH: Halle's RC model good for predicting the absence of toxicity

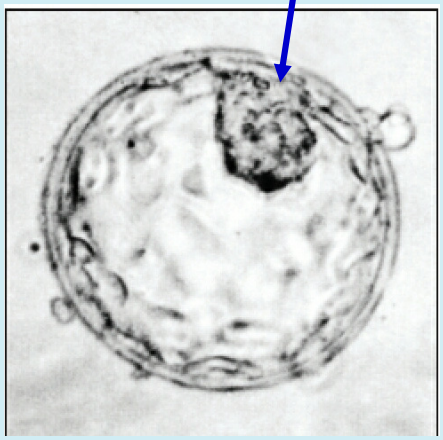



| New Chemicals: Oral toxicity distribution | |
|---|---|
| $LD_{50}$ [mg/kg] | chemicals [%] |
| ≤ 25 | 0 |
| > 25 - 200 | 3.1 |
| >200 - 2000 | 21.1 |
| >2000 | 75.8 |

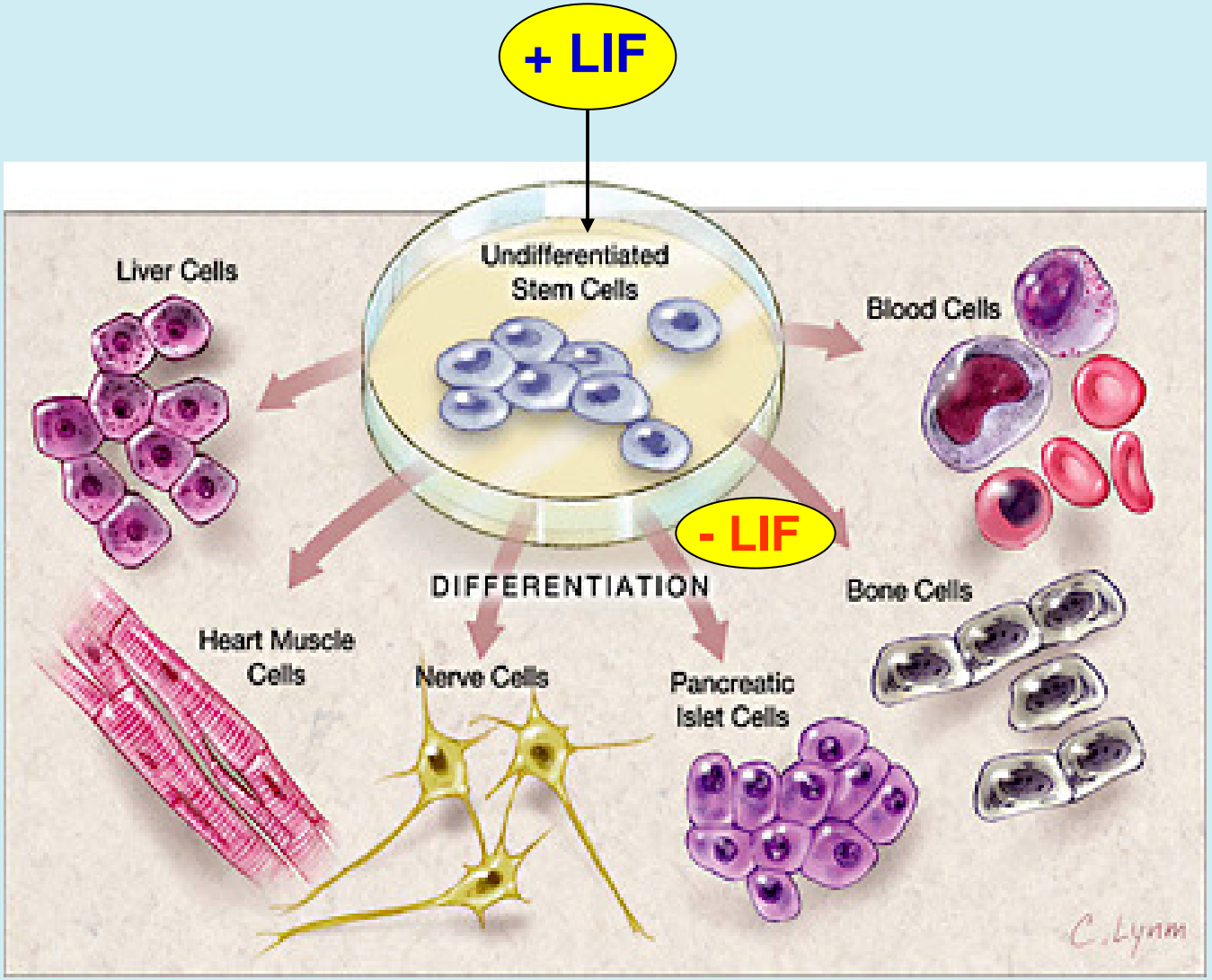
In these 2 classes 97% of all new industrial chemicals

# Origin and Differentiation Potential of mESC

Inner Cell Mass



Mouse Blastocyst



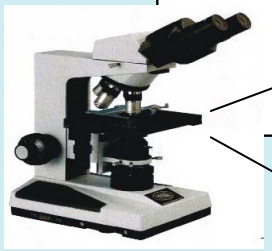
# Endpoints of the Embryonic Stem Cell Test

*Embryonic Stem Cells* *Differentiated Cells*

**Endpoint 1:**

**inhibition of differentiation**

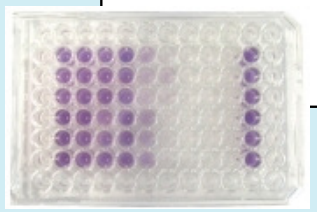
**ES cells**



**Endpoint 2:**

**cytotoxic effects**

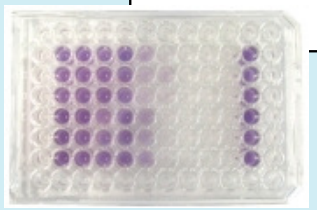
**ES cells**



**Endpoint 3:**

**cytotoxic effects**

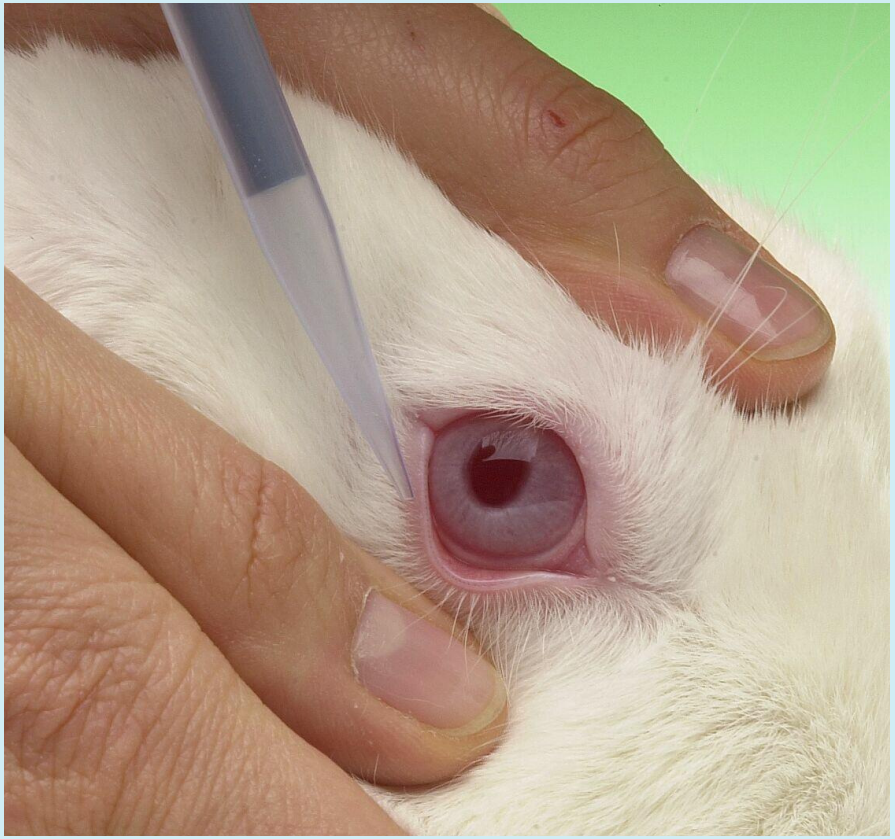
**3T3 cells**

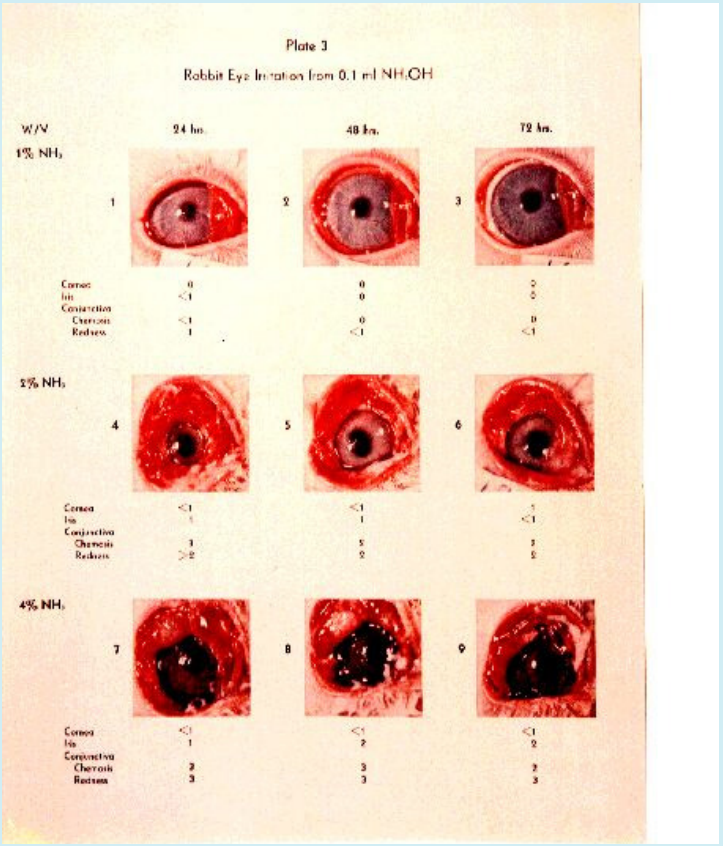


## Endpoints: assessment from concentration response curves

1. inhibition of differentiation in ES cells → $ID_{50}$
2. cytotoxic effects on ES cells → $IC_{50}$ D3
3. cytotoxic effects on 3T3 cells → $IC_{50}$ 3T3

# Draize Rabbit Eye Irritation Test






**Draize rabbit‘s eye test**

**Rabbit eyes exposed for 72 hrs.**

**PRINZIP:** Applikation von 100mg/100 µl Testsubstanz in das Kaninchen

**BEOBACHTUNG:** bis zum Abklingen der Symptome (max. 3 Wochen)

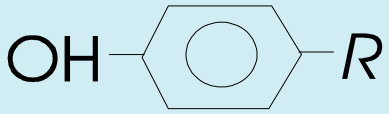
**BEURTEILUNG:** CORNEA *Trübung, ggf. Fläche*

IRIS

KONJUNKTIVA *Rötung, Schwellung ggf. Tränenfluß*

## Structural Rules

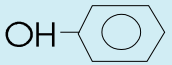
Example:

IF  R = aliph. chain

THEN "corrosive"

Explanation:

"Corrosive" according to EU-legislation* are:

 Phenol (R34)

 Kresol (R34)

* Directive 92/32/EEC amending for the seventh time Directive 67/548/EEC on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances. O.J. L154 5th of June 1992.

# (Q)-SAR quantitative Structure Activity Relationship

ECB QSARs

ihcp EUROPEAN COMMISSION Joint Research Centre

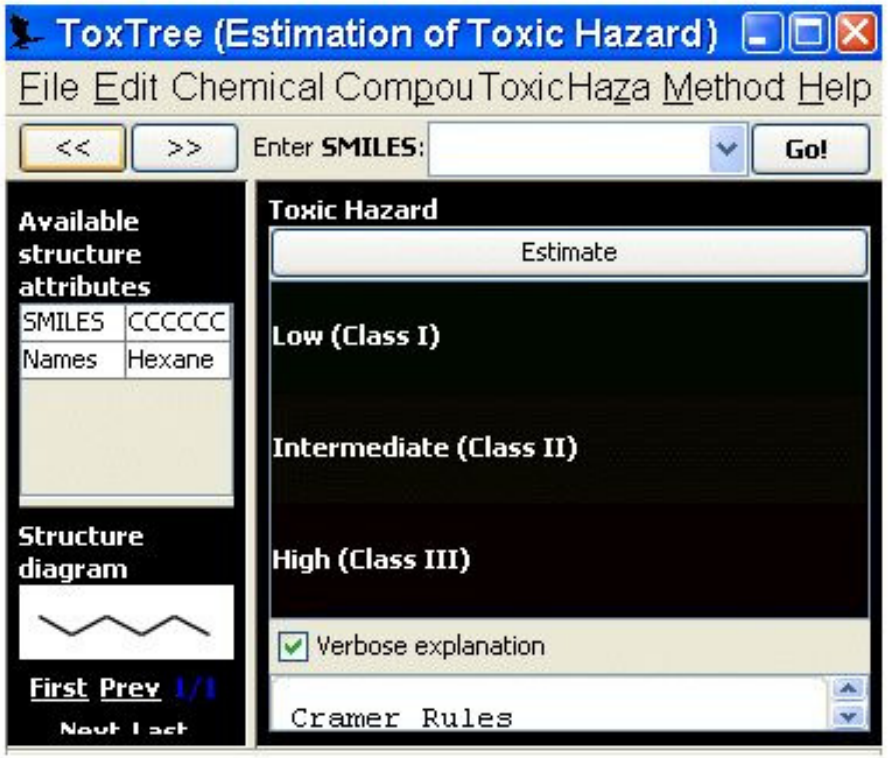
ECB Home Page | Documents | About the Group | Background | Events | Information Sources | Publications | QSAR Tools

- sessment of Chemicals
- ocides
- isting Chemicals
- port-Import
- w Chemicals
- mputational Toxicology
- ACH
- assification & Labelling
- sting Methods
- ACH-IT & Informatics
- CLID 5
- IS
- FOCAP
- ntacts
- cumentation
- gislation
- ks
- wsletter

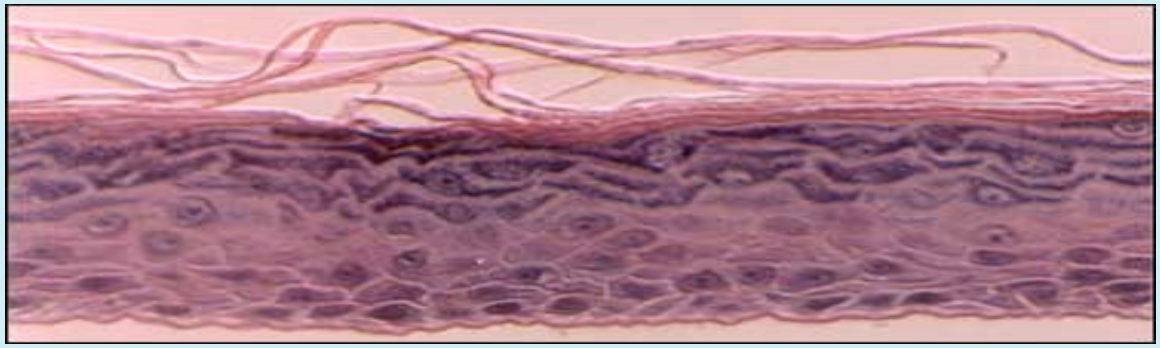
## Toxtree

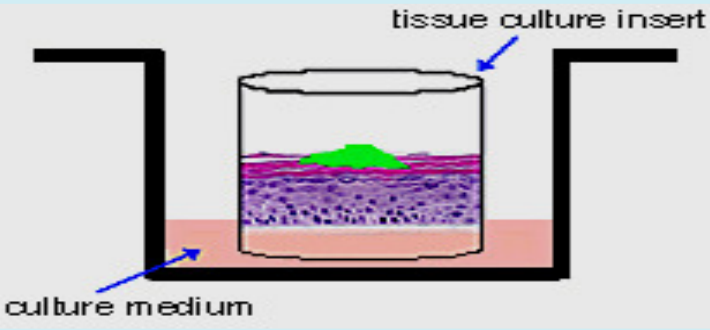
Toxtree is a flexible and user-friendly open-source application that places chemicals into categories and predicts various kinds of toxic effect by applying decision tree approaches, such as the Cramer classification scheme, the Verhaar scheme for aquatic modes of action, and a rulebase for skin irritation and corrosion based on rules developed by the German Federal Institute for Risk Assessment (BfR) and collaborators.

Toxtree was developed by IdeaConsult Ltd (Sofia, Bulgaria) under the terms of an ECB contract. The software is made freely available by ECB as a service to scientific researchers and anyone with an interest in the application of computer-based estimation methods in the assessment of chemical toxicity.



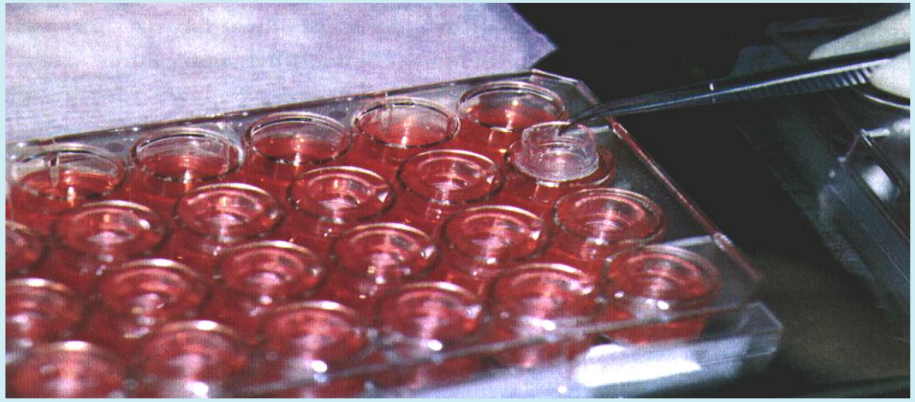
# human 3-D skin models

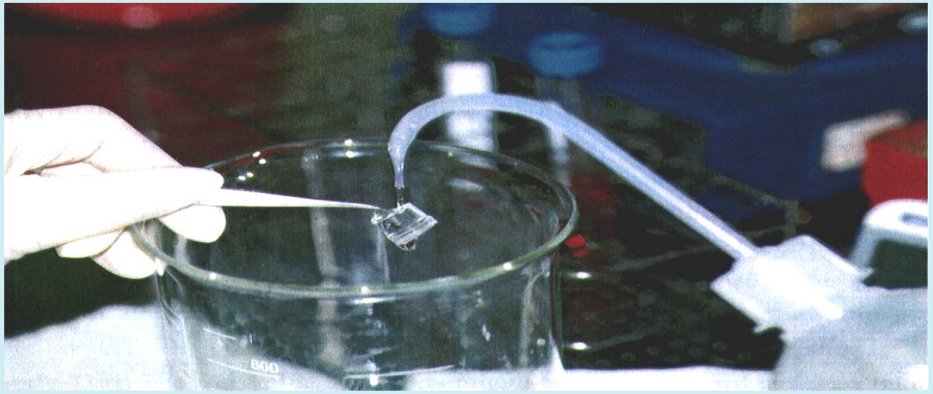





**EpiDerm, MatTek, USA**

**SkinEthic, Frankreich**





**... are easy to handle...**

ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT

# OECD GUIDELINE FOR THE TESTING OF CHEMICALS

## DRAFT PROPOSAL FOR A NEW GUIDELINE

## *In Vitro* Skin Irritation: Reconstructed Human *Epidermis* (RhE) Test Method

## INTRODUCTION

1. Skin irritation refers to the production of reversible damage to the skin following the application of a test substance for up to 4 hours [as defined by the United Nations (UN) Globally Harmonized System of Classification and Labelling of Chemicals (GHS)](1). This Test Guideline provides an *in vitro* procedure that, depending on country requirements, may allow determining the skin irritancy of chemicals as a stand-alone replacement test, as a screen, or within a testing strategy in combination with, if appropriate, a weight of evidence approach.

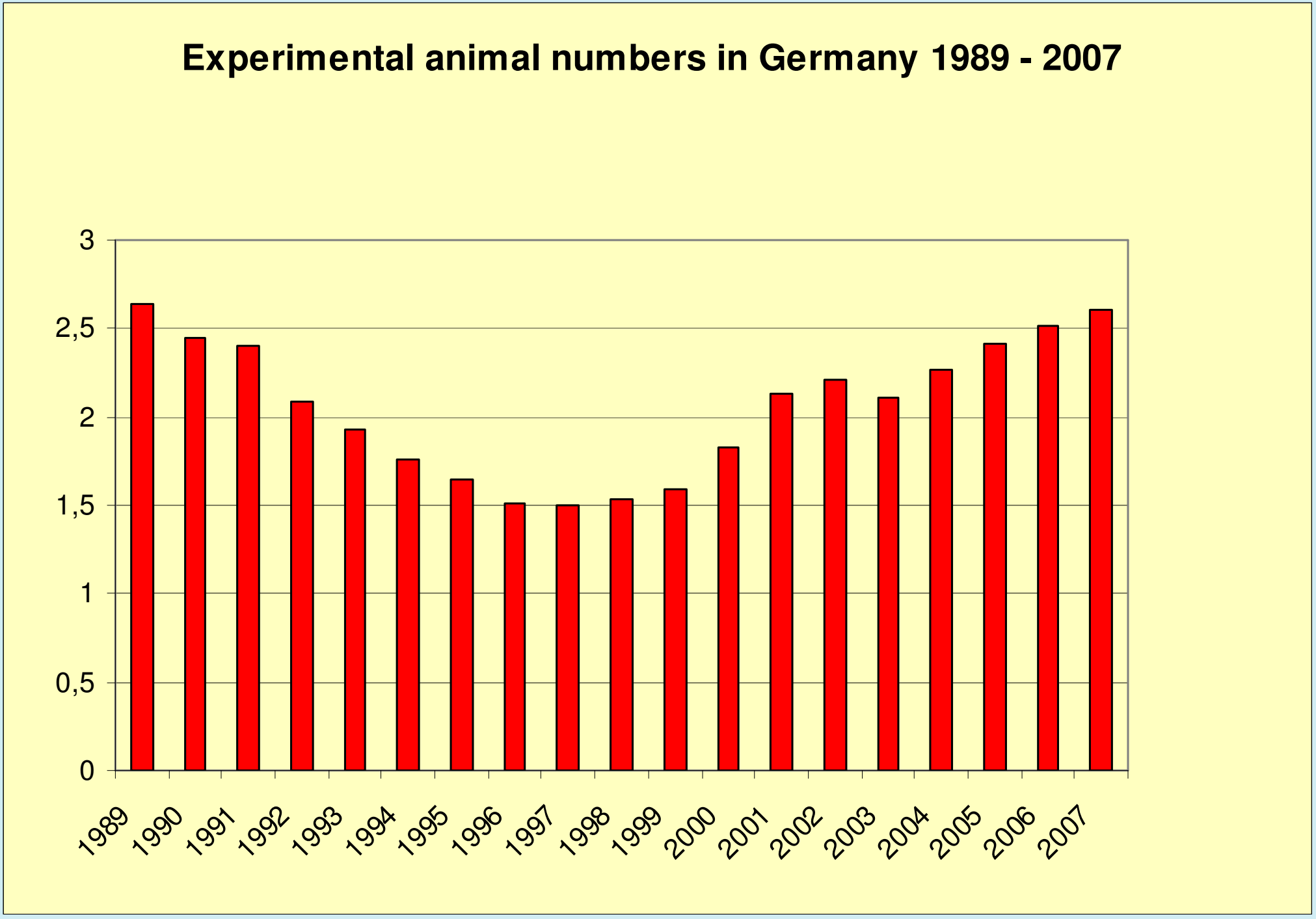
# ZEBET's management of validation studies 1992-2009

- ECVAM / COLIPA / ZEBET validation project of *in vitro* phototoxicity tests *1992-1998*
  ➔ 3T3 NRU in vitro phototoxicity test **accepted 2000**
- ECVAM "catch up" study on commercial skin models for corrosivity testing *1997-1998*
  ➔ Epiderm® **accepted 2000**
- ECVAM validation study of 3 *in vitro* embryotoxicity tests: WEC, MM, EST *1997-2000*, **successful**
- ECVAM study on the use of skin models for phototoxicity testing *1996-2000,* **successful**
- BMBF study on improving the embryonic stem cell test EST: in vitro embryotoxicity test ➔ **molecular markers**
- BMBF prevalidation study on the use of human skin models for skin penetration testing ➔ **2002-2007 successful**
- ECVAM validation study of in vitro skin irritation tests applying human skin models ➔ **2004-2007 successful**
- BMBF in vitro DNT (developmental neurotox) ➔**begin 2007**

# ZEBET/BfR initiatives to reduces animal testing in specific fields of regulatory toxicology

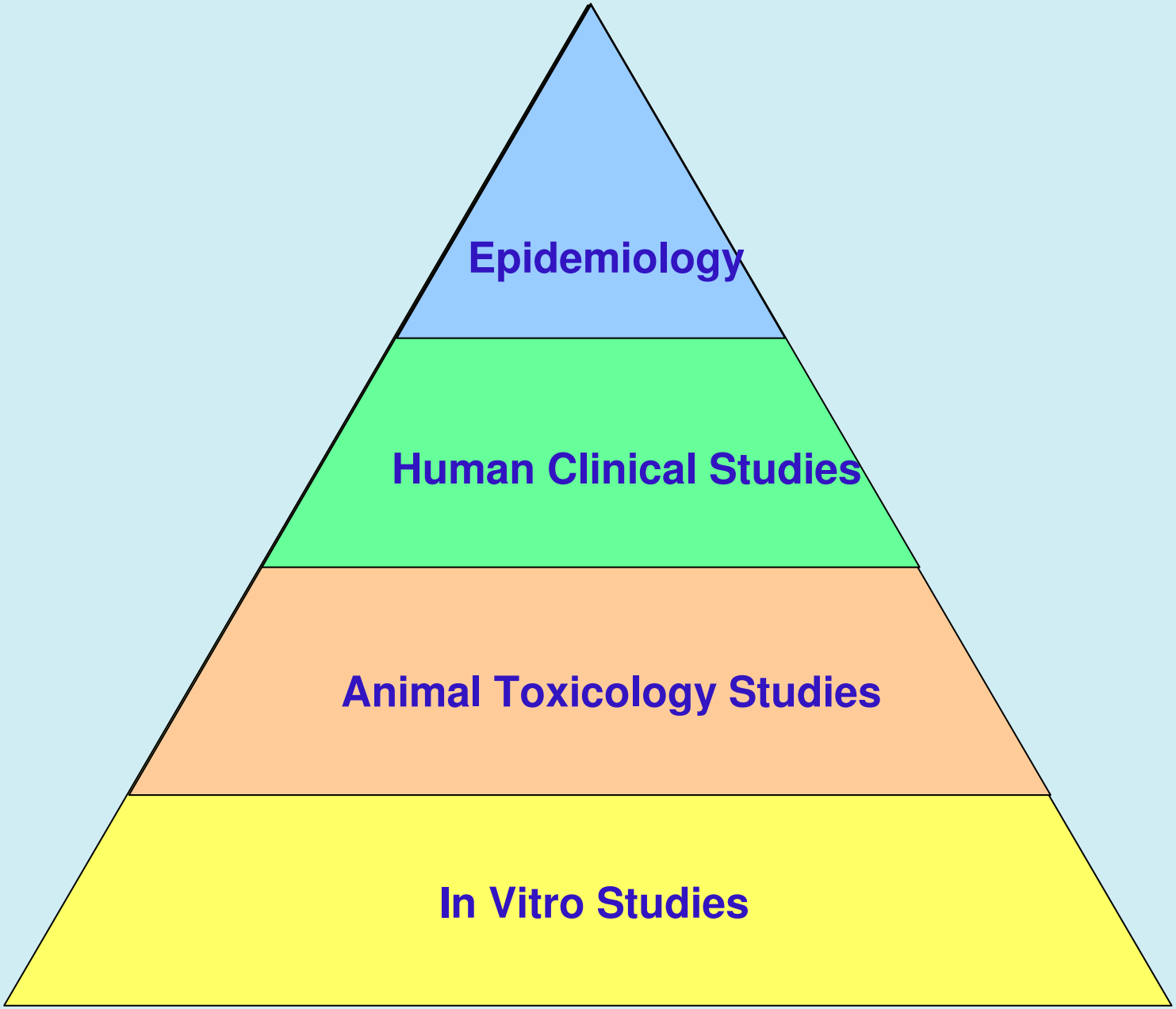
- **Agrochemicals/Pesticides**
  - ➔ **The use of the dog as a second species in pesticides testing**
  - ➔ **BfR, SET & German agrochemical industry initiative 1995-2003**
  - ➔ **supported by EPAA 2009**
- **Food safety**
  - ➔ **replacing the mouse bioassay for shellfish toxins**
  - ➔ **ZEBET/BfR activity**
  - ➔ **2009 accepted by EFSA**
- **Safety of biologicals**
  - ➔ **replacing the LD-50 test for botulinum toxin**
  - ➔ **BfR & BfArM initiative 2009**
  - ➔ **international BoNT EWG established 2009**

# The challenge for the 3Rs movement 2009:
# How are we going to cope with rising animal numbers ??



# In Vitro Studies: What is their Role in Toxicology?

Robert Devlin US EPA 2005



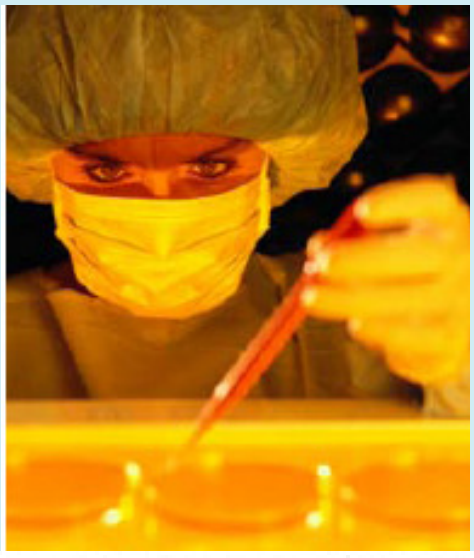
2007

THE NATIONAL ACADEMIES

REPORT IN BRIEF

# Toxicity Testing in the 21st Century: A Vision and a Strategy

**Advances in molecular biology, biotechnology, and other fields are paving the way for major improvements in how scientists evaluate the health risks posed by potentially toxic chemicals found at low levels in the environment. These advances would make toxicity testing quicker, less expensive, and more directly relevant to human exposures. They could also reduce the need for animal testing by substituting more laboratory tests based on human cells. This National Research Council report creates a far-reaching vision for the future of toxicity testing.**

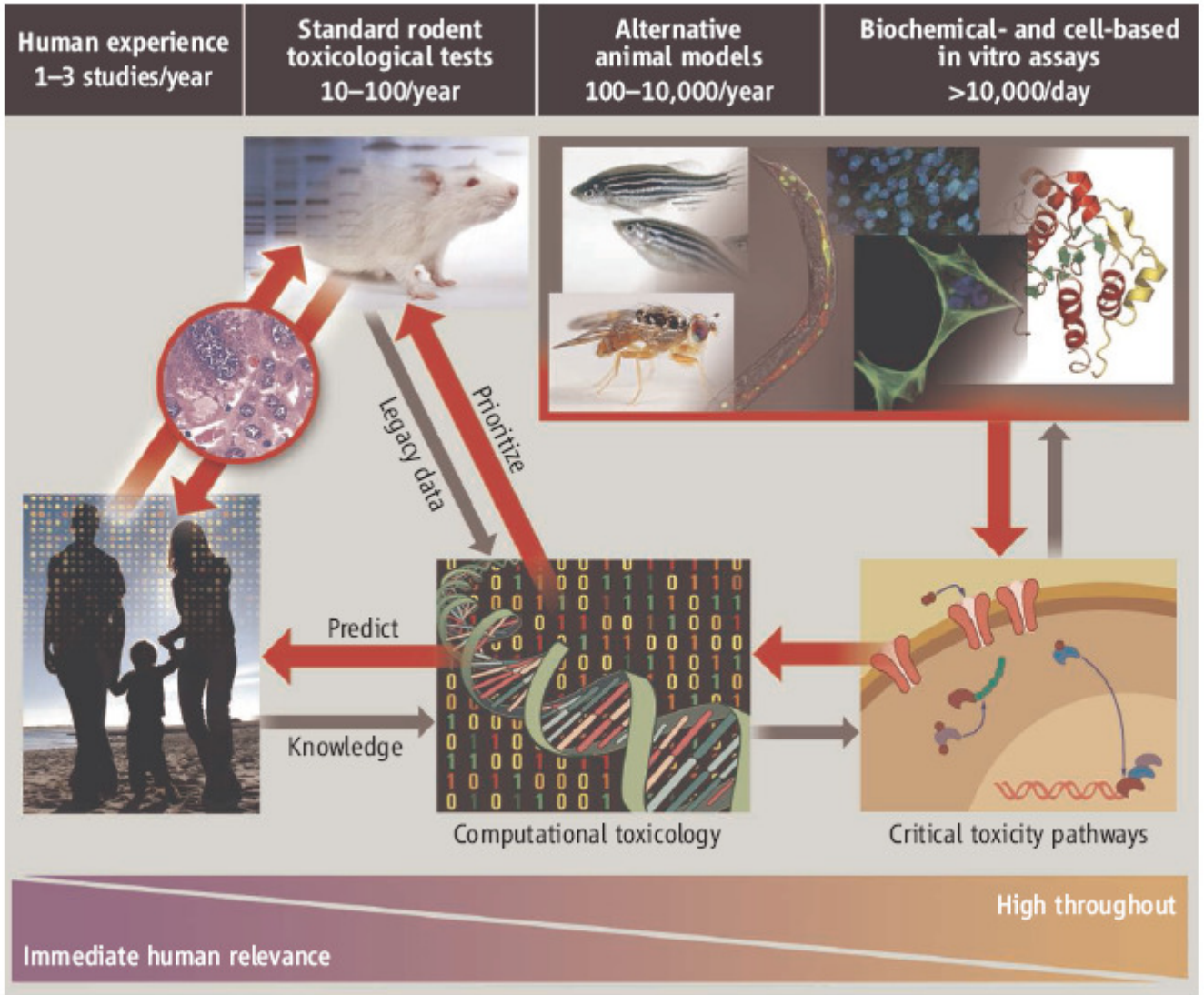


OXICOLOGY

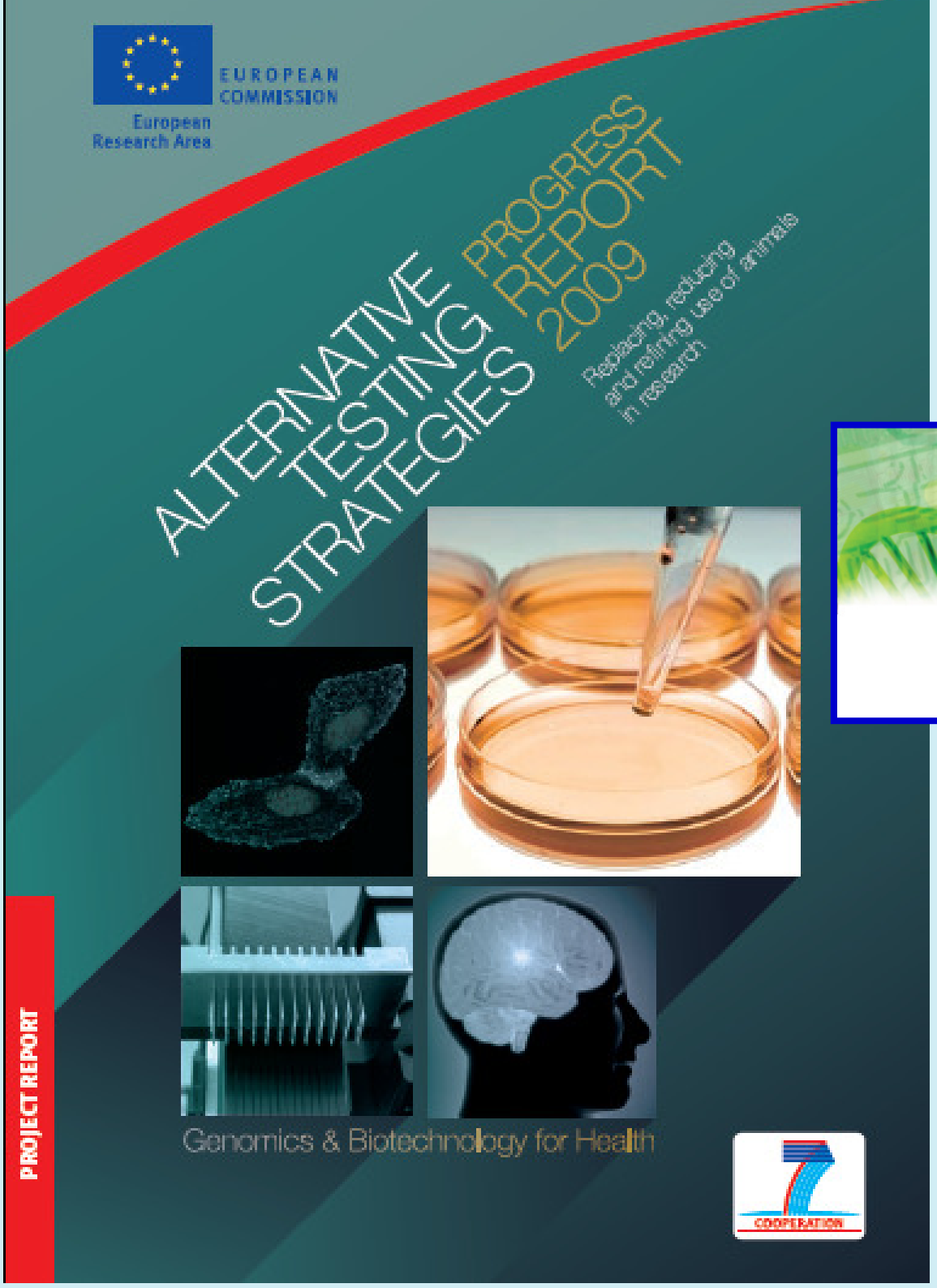
# Transforming Environmental Health Protection

rancis S. Collins,[1*†] George M. Gray,[2*] John R. Bucher[3*]

**We propose a shift from primarily in vivo anima studies to in vitro assays, in vivo assays with lower organisms, and computational modeling for toxicity assessments.**




**Transforming toxicology.** The studies we propose will test whether high-throughput and computational tox-




# FP7 Coordination project 2010-2013

## AXLR8

Accelerating the transition to a toxicity pathway-based paradigm for chemical safety assessment through internationally co-ordinated research and technology development

# Thank you for the support during the first 20 years of ZEBET

## Behind every success there are many dedicated supporters

## In Germany

The German animal welfare movement and the German Bundestag

BGA: Eberhard Bulling & Dieter Grossklaus

BgVV: The ZEBET Commission

BfR: Andreas Hensel & Reiner Wittkowski

ZEBET: Barbara Grune, Manfred Liebsch, Andrea Seiler
and a very dedicated staff

Ministries: BMG, BMVEL, BMBF

## Abroad

Colleagues from ECVAM, ICCVAM, JacVam, JSAAE, ESTIV, ERGATT

## Special thanks to Michael Balls (FRAME, ECVA & ATLA)

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