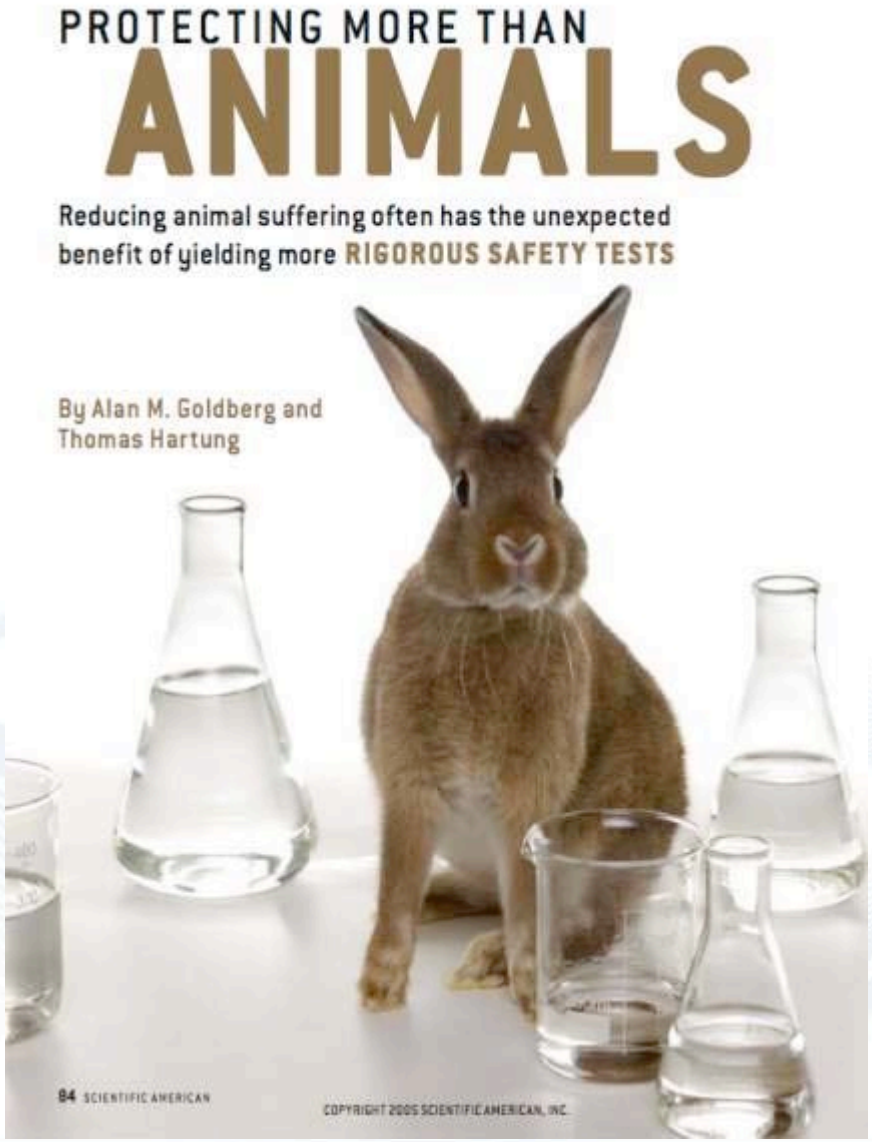
# *Opportunities for replacing animals in toxicity testing*

**Thomas Hartung**

**Doerenkamp-Zbinden Professor and Chair for Evidence-based Toxicology, EHS**
**Director, Center for Alternatives to Animal Testing (CAAT)**
**Joint appointment: Molecular Microbiology and Immunology**
**Bloomberg School of Public Health, Johns Hopkins University, Baltimore, US**

**Professor of pharmacology and toxicology, University of Konstanz, Germany**

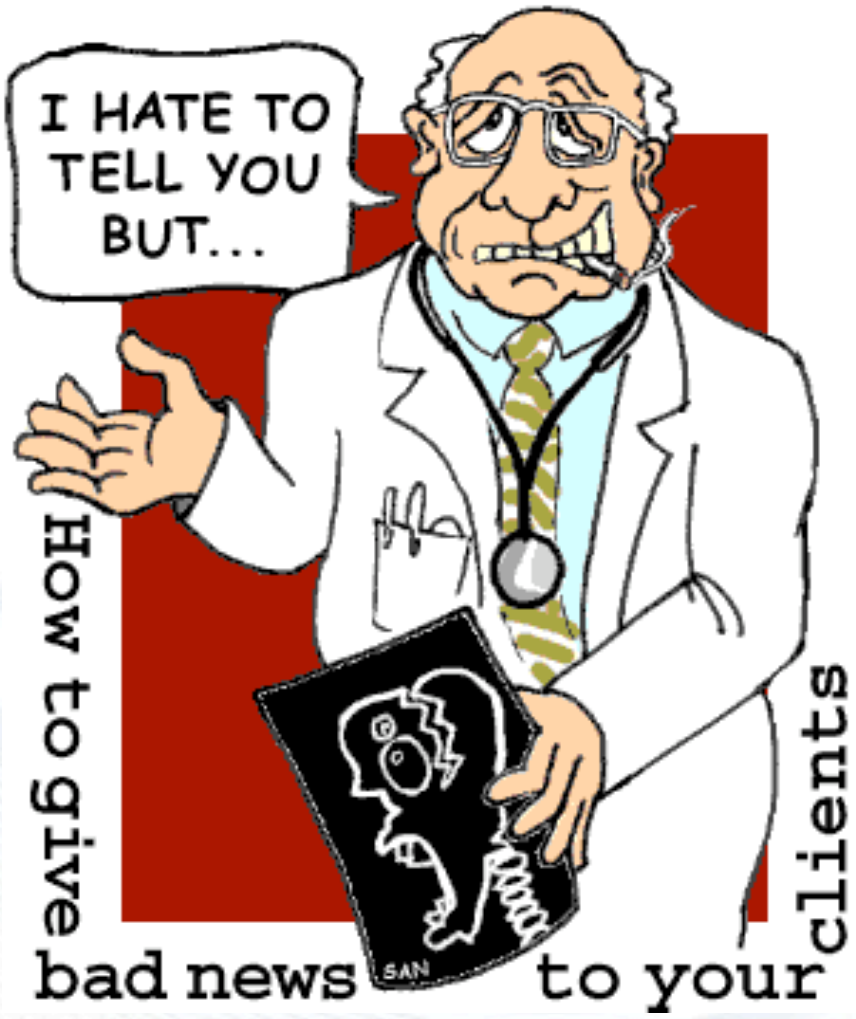



- We need all tools in the life sciences, but all tools have limitations
- Animal welfare is not the only reason for alternatives
- The value of animal test results is overestimated
- 3Rs (reduce, replace, refine) as societal compromise

# Not to forget:

## We can also do a lot for reduction and refinement

## Pharma Reduction & Refinement Work Group




## *Traditional 3Rs methods will not be the solution to the problem*

- **Little perspective for complex endpoints**
- **2/3 fail validation**
- **hardly solved the cosmetics 7th amendment challenge for 2009, no way for 2013**

# 2004-2009

**(www.reprotect.eu)**

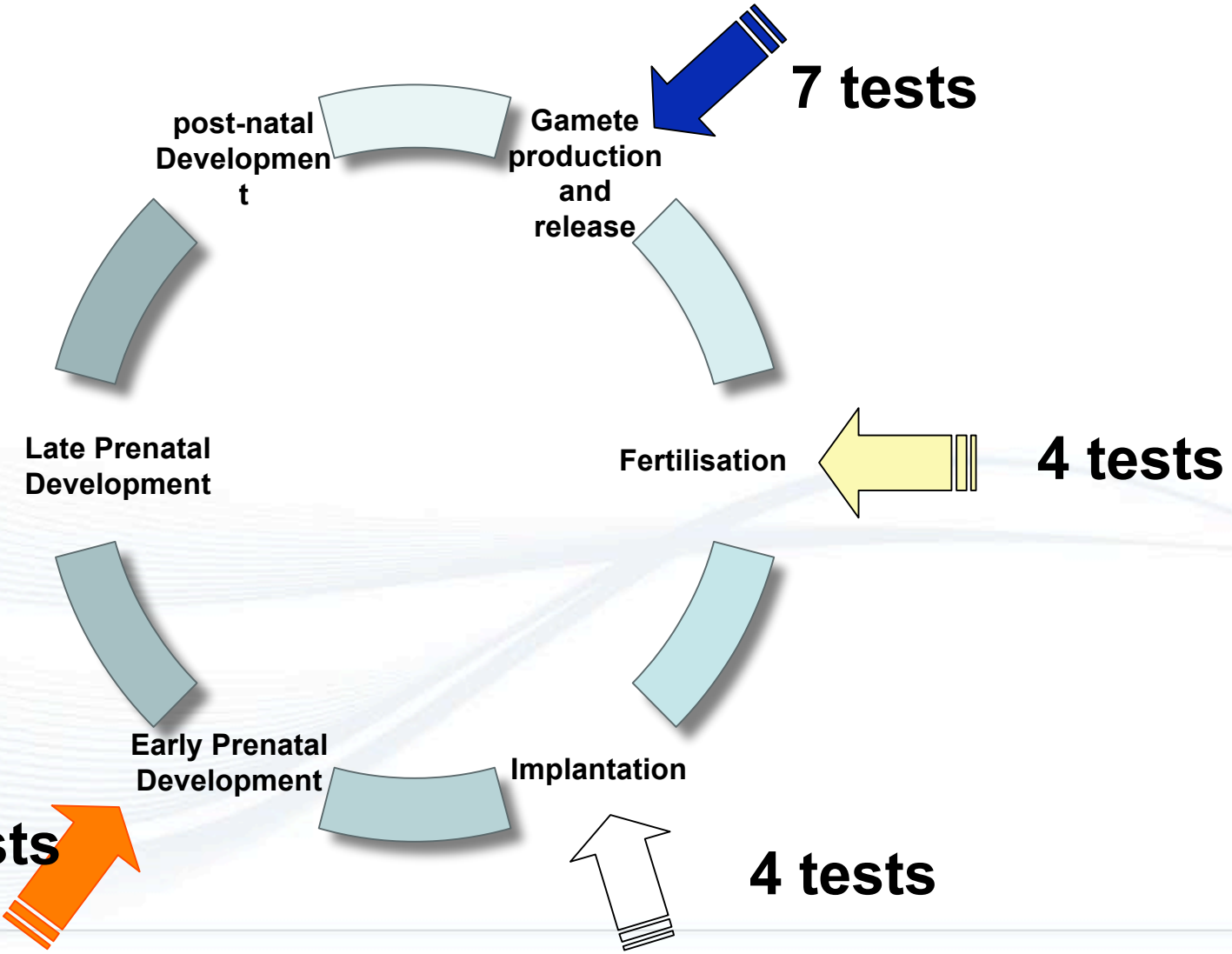
***LSHB-CT-2004-503257***

Development of a novel approach in hazard and risk assessment of reproductive toxicity by a combination and application of *in vitro*, tissue and sensor technologies

32 participating groups

# ReProTect

## Reproductive cycle



# The ReProTect Feasibility Study

**Effect:**
**Positive (red)**
**or negative (green)**

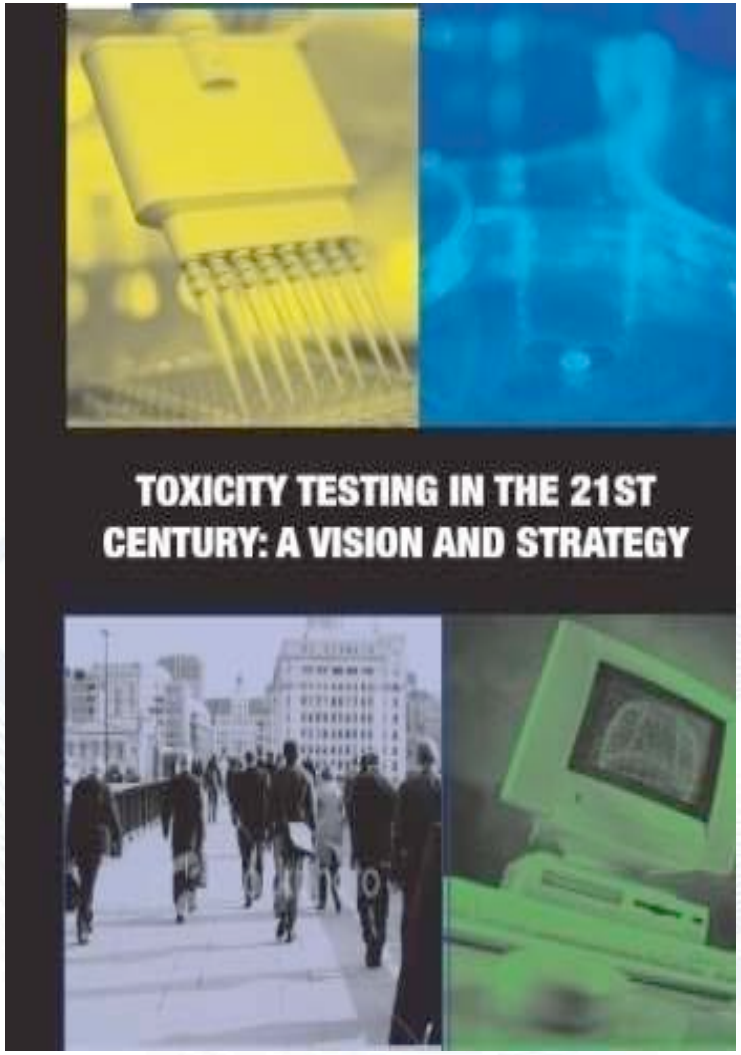
√ **Correctly predicted**

(√) **Partly predicted**

x **Not correctly predicted**

| Chemical | Female fertility | Male fertility | Developmen-tal toxicity |
|---|---|---|---|
| 1 | √ | √ | √ |
| 2 | √ | √ | √ |
| 3 | x | x | √ |
| 4 | √ | √ | √ |
| 5 | √ | √ | √ |
| 6 | √ | √ | √ |
| 7 | √ | x | √ |
| 8 | √ | x | √ |
| 9 | √ | √ | √ |
| 10 | (√) | (√) | (√) |

# NAS vision report Tox-21c




- An atmosphere of departure in toxicology
- Lessons learned from alternative methods and their validation
- New technologies from biotech and (bio-)informatics revolution
- Mapping of pathways of toxicity (PoT)

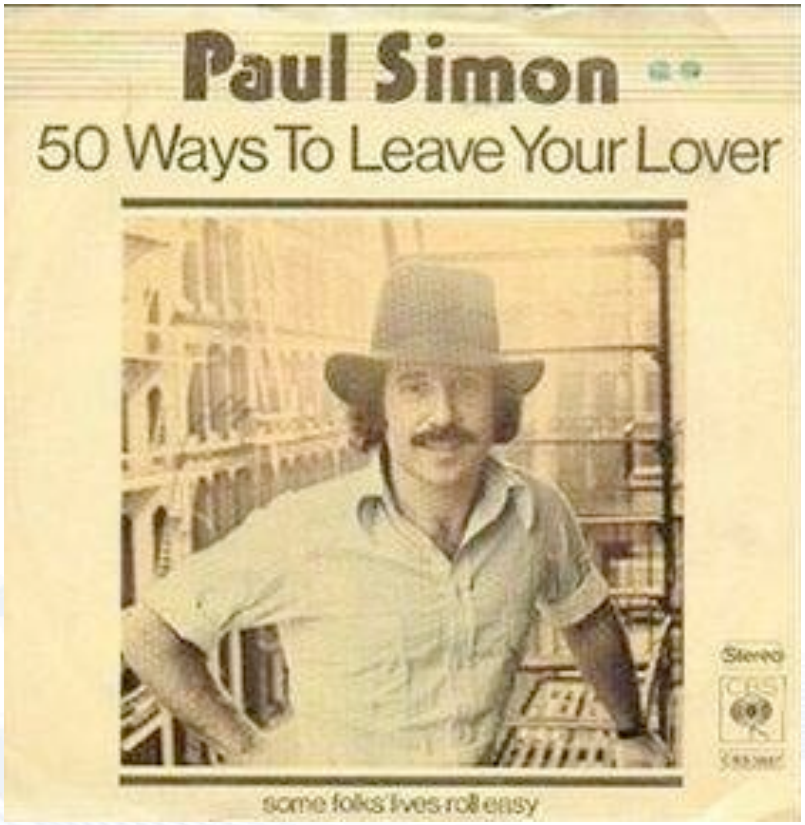
# *Friday 15th Feb 2008: Coalition of EPA, NTP and NIEHS-CGC to implement NRC vision [Science 2008, 319:906-7]*

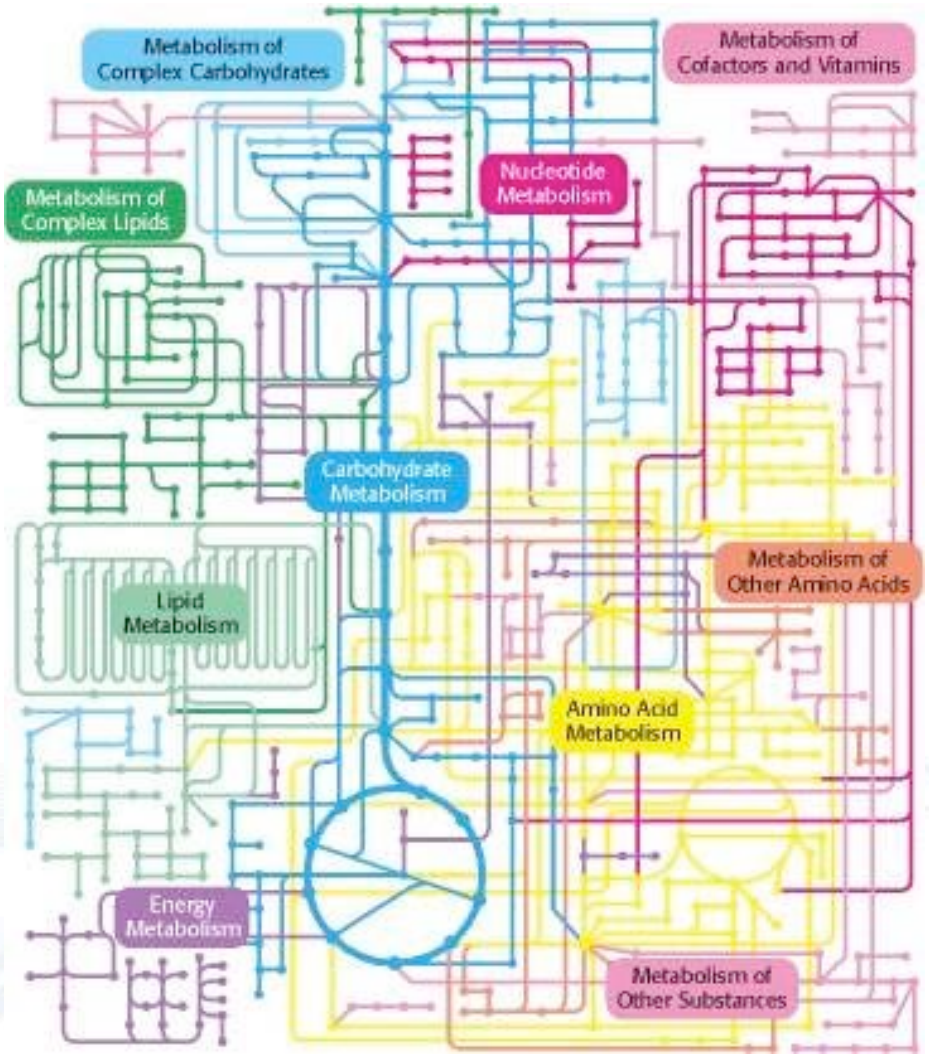
- **"We propose a shift from primarily in vivo animal studies to in vitro assays, in vivo assays with lower organisms, and computational modeling for toxicity assessments"**
- **"[toxicity testing] was expensive, time-consuming, used animals in large numbers and didn't always work" Francis Collin, now Director NIH**
- **"Animal testing won't disappear overnight, but the agencies' work signals the beginning of an end." Elias Zerhouni, at the time Director NIH**

**quotes: USA TODAY**

Hamburg, M.A. (2011). Advancing regulatory science. *Science* 331, 987

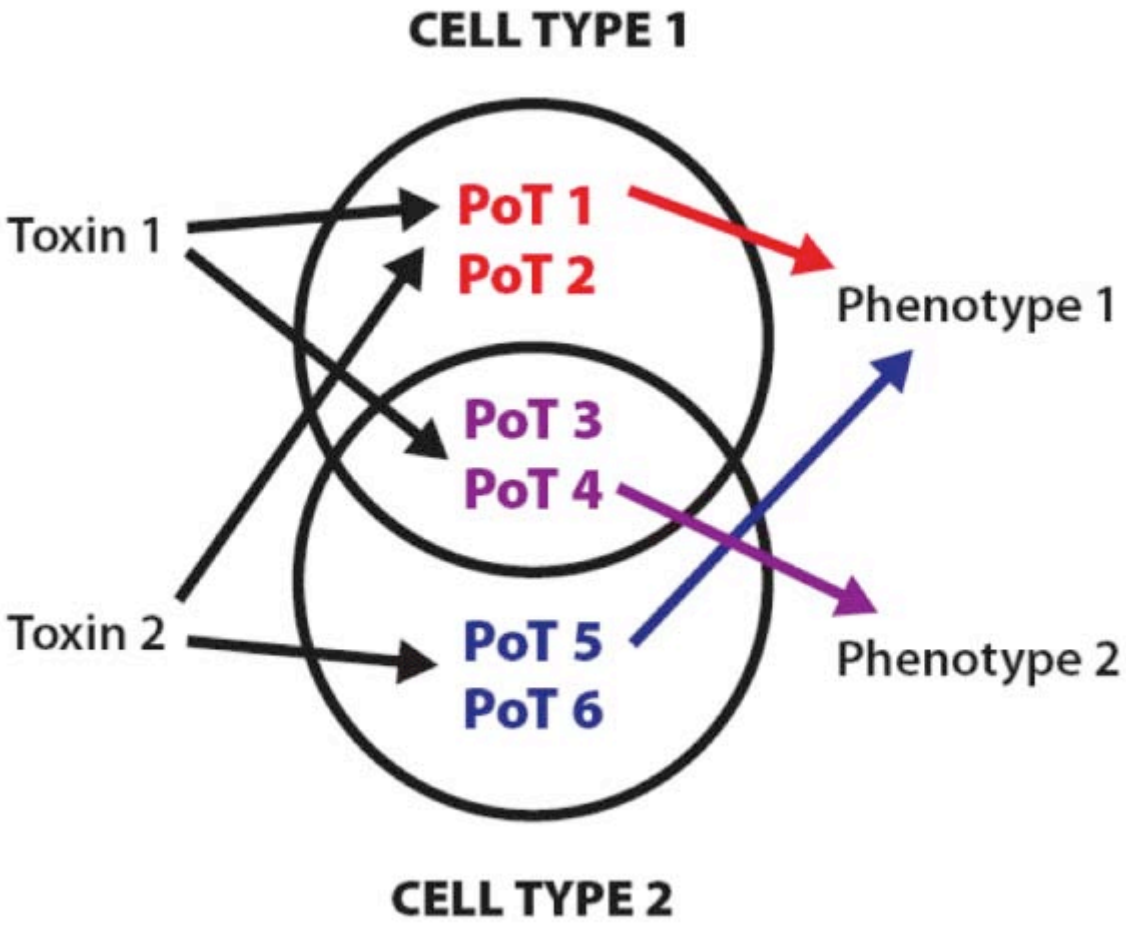
***"We must bring 21st century approaches to 21st century products and problems. Toxicology is a prime example. Most of the toxicology tools used for regulatory assessment rely on high-dose animal studies and default extrapolation procedures and have remained relatively unchanged for decades, despite the scientific revolutions of the past half-century. We need better predictive models to identify concerns earlier in the product development process to reduce time and costs. We also need to modernize the tools used to assess emerging concerns about potential risks from food and other product exposures. … With an advanced field of regulatory science, new tools, including functional genomics, proteomics, metabolomics, high-throughput screening, and systems biology, can replace current toxicology assays with tests that incorporate the mechanistic underpinnings of disease and of underlying toxic side effects. This should allow the development, validation, and qualification of preclinical and clinical models that accelerate the evaluation of toxicities during drug development."***



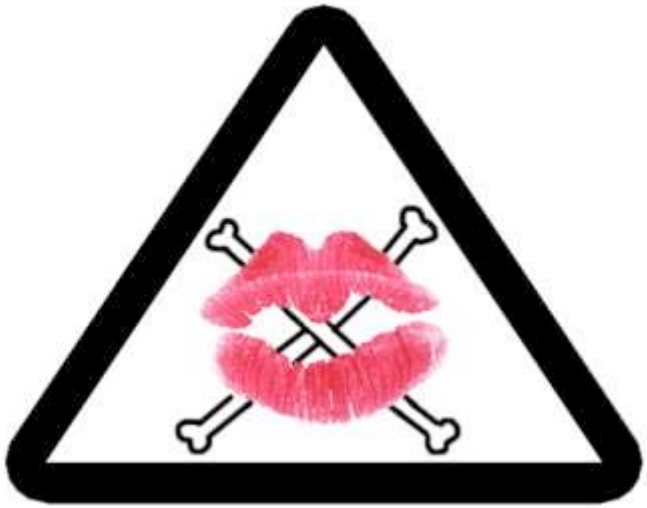




**…and a couple of hundred ways to kill a cell**

# *Mapping the (finite number of) pathways of toxicity*




**Annotation to:**
**-Hazard**
**-Toxin (class)**
**-Cell type**
**-Species**

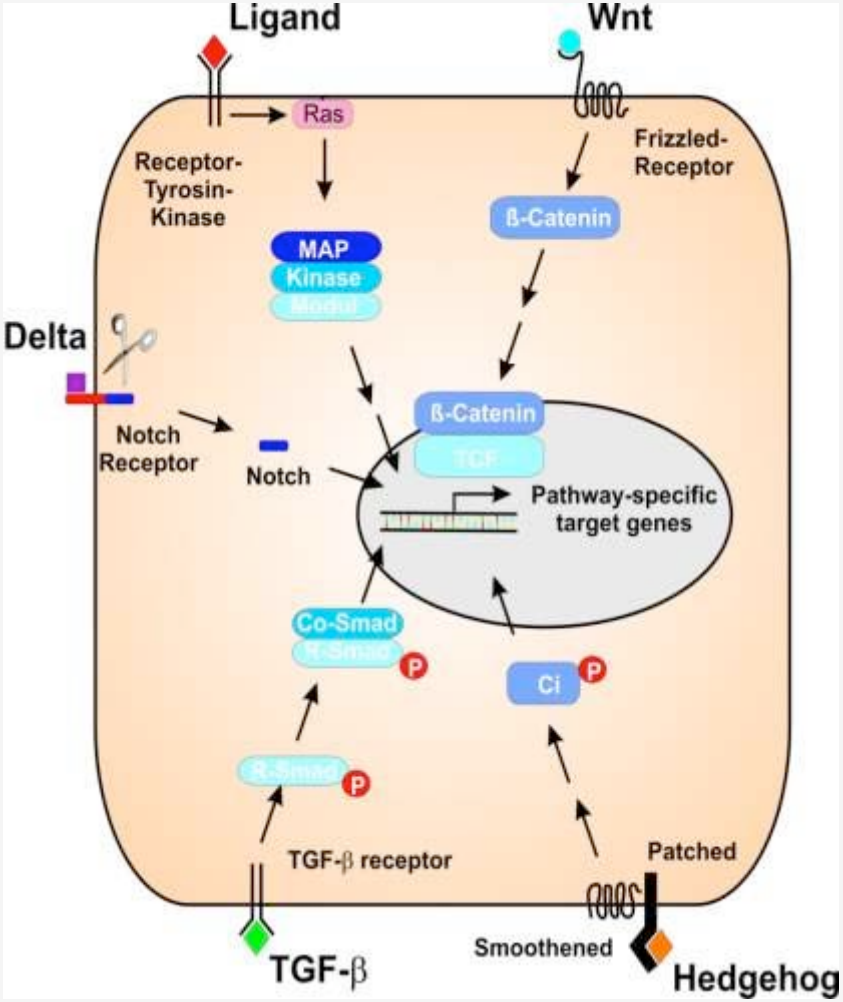


*We need to find (concentrations of) substances, which trigger no PoT*

**Most chemicals are not toxic:**

- **90% not acutely toxic**
- **97% not skin corrosive**
- **93% not skin irritant**
- **97% not teratogenic**
- **80-95% not carcinogenic**
- **80% not eye irritating**
- **65% not skin sensitizing**

# *Five signalling pathways are important during early development*

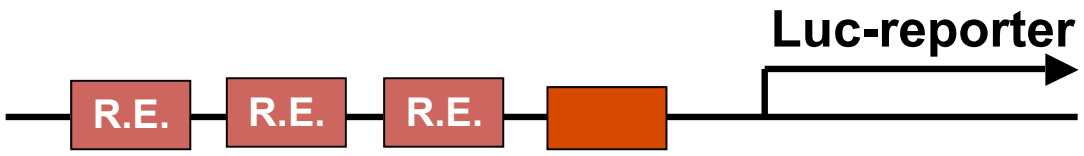



**for review see: Scientific frontiers in Developmental Toxicology and Risk Assessment, Nat. Acad. Press, 2000**

# *The ReproGlo assay*

## Luciferase-reporter based detection system

**Stable transfection of reporters into mES cells**




**Uibel et al. Reprod. Tox. 2010**

**Correctly identified human reproductive toxicants:**

- -Lithium chloride
- -Retinoic acid
- -Potency of different Valproic acid derivatives
- -(with metabolizing system) cyclophosphamide

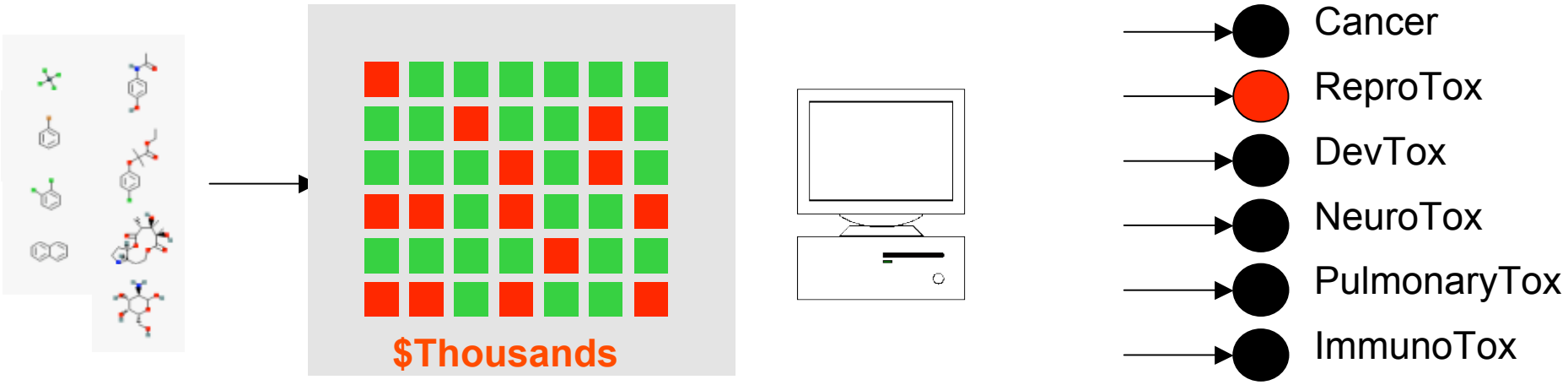
**In collaboration between ECVAM and Prof. M. Schwarz, Tübingen, Germany**

From R. Kavlock, EPA, 2010

# ToxCast Bioactivity Profiling

United States Environmental Protection Agency

*in vitro* testing  *in silico* analysis




HTS
*-omics*

Bioinformatics/
Machine Learning



Dix *et al*, Tox Sci 95:5-12 (2007)
www.epa.gov/ncct/toxcast

From R. Kavlock, EPA, 2010

# Deepwater Horizon

## Oil Exploration Platform Explodes April 20, 2010

- Estimated 4.9 million barrels of South Louisiana Crude released

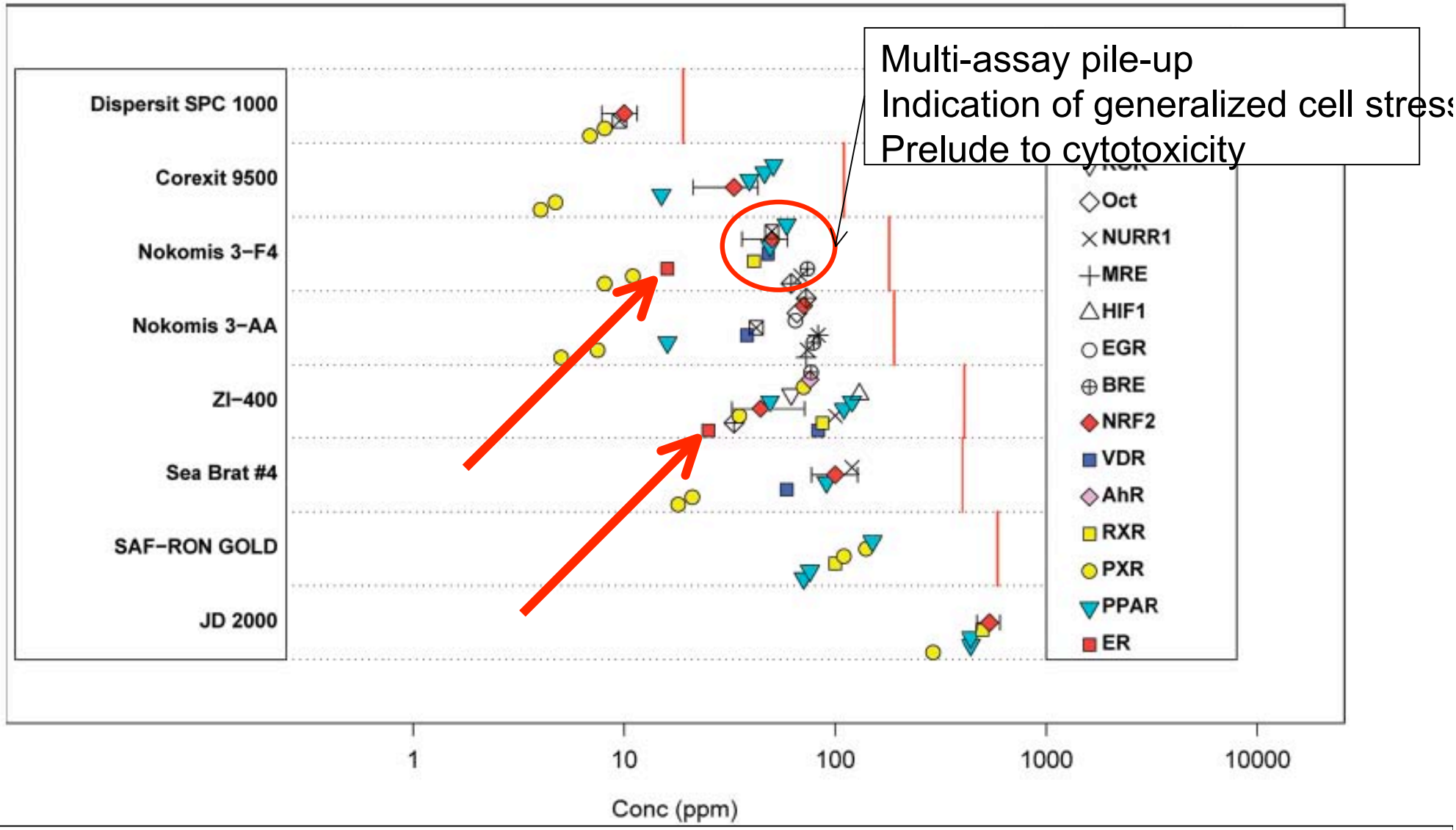
## 1.8 million gallons of dispersant used

- 1072K surface; 771K subsea
- Corexit 9500A (9527 early in spill)

## EPA Administrator call for less toxic alternative

- Verification of toxicity information on NCP Product Schedule
- ORD involvement in assessments of dispersant toxicity

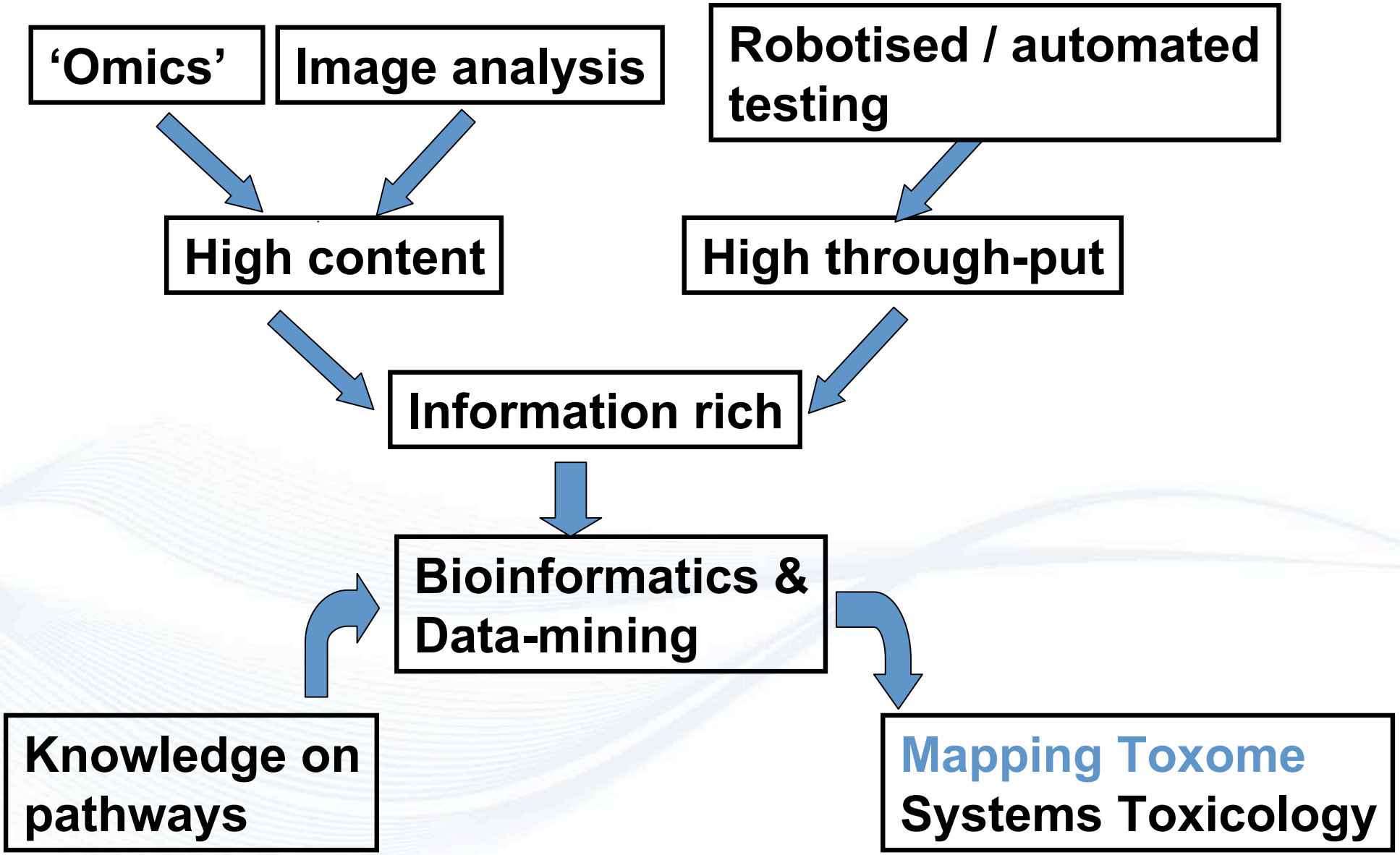
# Dispersant Transcription Factor Profiling




Little specific activity seen except for PXR/ PPAR - consistent with xeno-sensing

**From R. Kavlock, EPA, 2010**

Judson et al , ES&T, 2010



# *Proof of principle: Metabolic profiling and PoT identification in DNT*

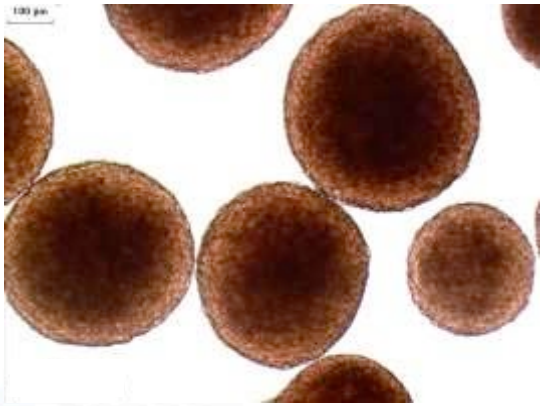
- **DNT (autism, hyperactivity); animal test $1.4 million, 1400 animals per substance**

- **Identify metabolite changes relevant for neurodevelopment with reference compounds**

- **Agilent thought leader award: LC/MS system**

- **Integrate with genomics**

# *Principal component analysis of metabolomics*

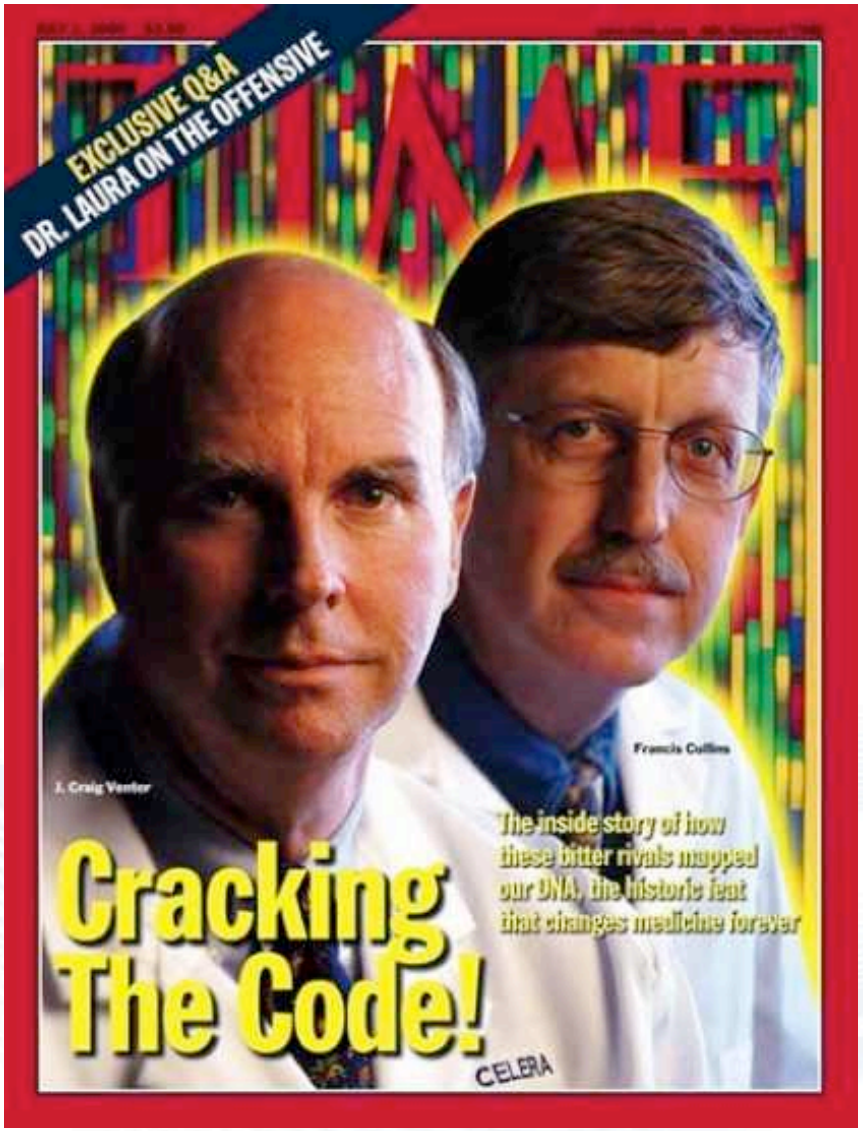


Rat
"mini-brains"

lead

TCE

PoT identification




# *Lessons learned from*

HUGO

**-Really took off when there was private competition**
**-Celera became "acceptable" only when feeding into public database**



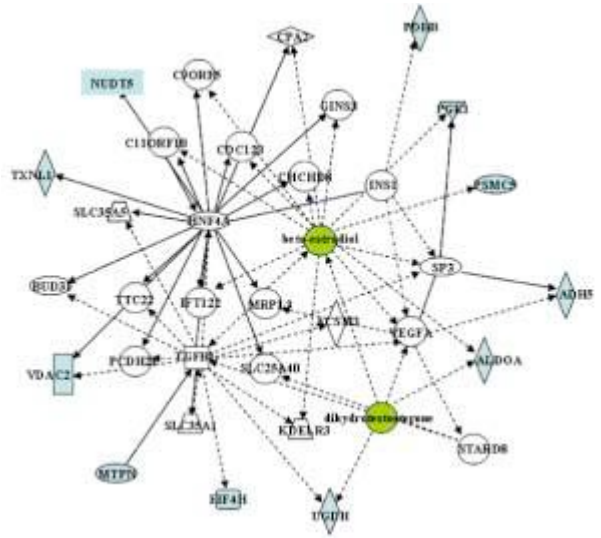
# PoToMaC - The Pathways of Toxicty Mapping Center

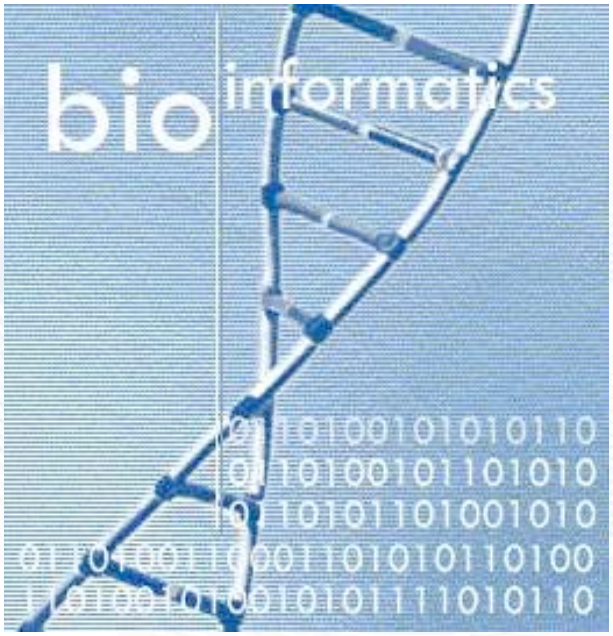
JOHNS HOPKINS BLOOMBERG SCHOOL of PUBLIC HEALTH

## *Starting point: (Pre-)Validated Alternatives*

**Robust protocols, good cell models**
**Regulatory acceptance**
**Available reference substances**
**Thresholds of adversity defined**
**$300 million of research & validation spent**

a) **3T3 fibroblasts** (acute toxicity, cancer, (phototoxicity)
b) **Human artificial skin models** (skin irritation and corrosion, genotoxicity, phototoxicity and skin penetration)
c) **Human blood monocytes** (inflammation, skin sensitization)
d) **MCF-7 cells** (endocrine disruption)
e) **HepaRG cells** (liver toxicity)
f) **Human blood lymphocytes** (genotoxicity)






# *PoT identification challenges*

- Number, variants
- Non-linear pathwyas
- Combination of various technologies beyond metabolomics and genomics (e.g. phosphroylation of G-proteins)
- Annotation
- Validation
- Governance of public database



- No concept for PoT mapping, validation
- No definition of adversity
- Various additional components needed for use of PoT
- Transition from current system

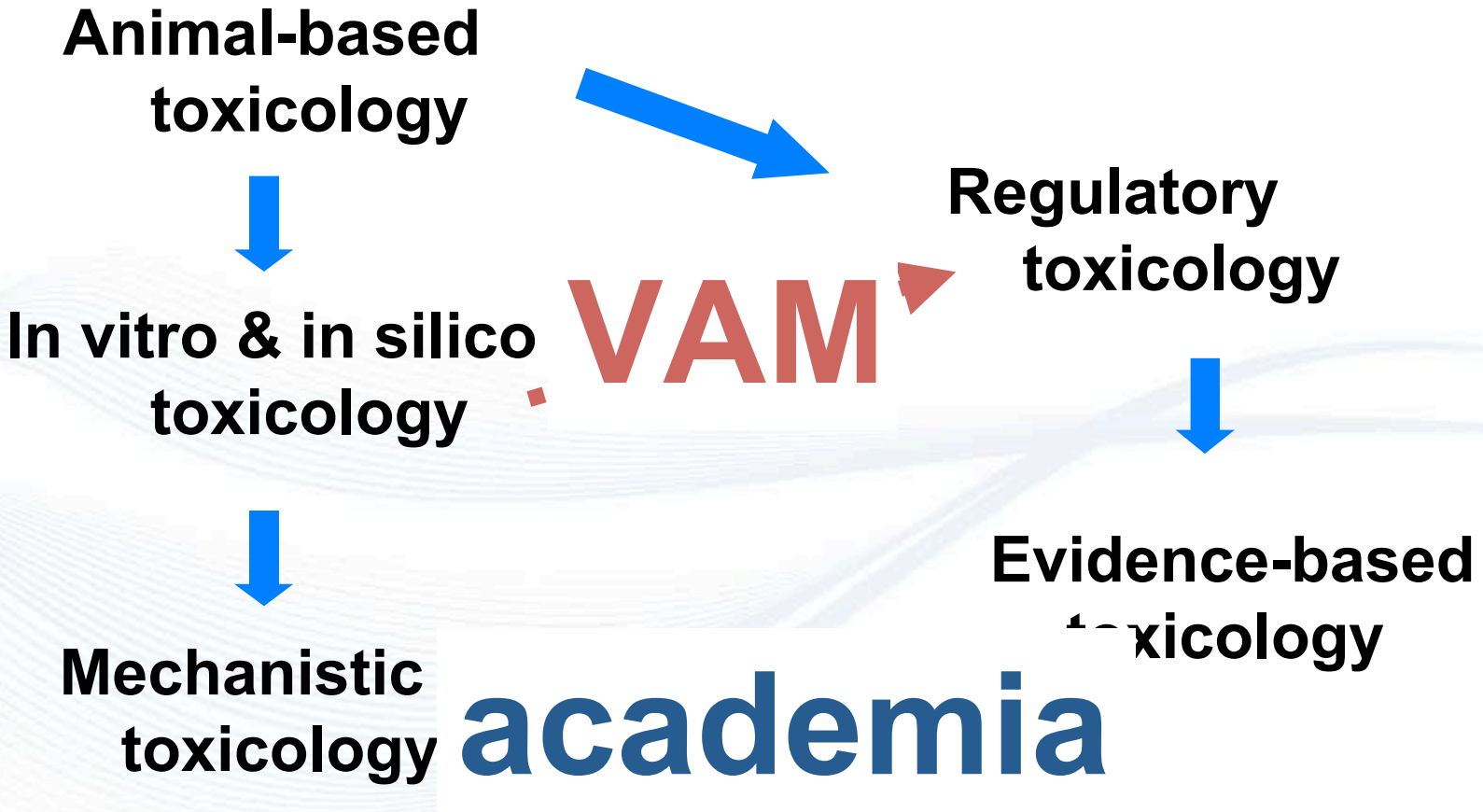
⇒ Evidence-based toxicology

CAAT Johns Hopkins University Center for

# Food for Thought ... on the Evolution of Toxicology and the Phasing out of Animal Testing

*Thomas Hartung*[1] *and Marcel Leist*[2]
[1]EC Joint Research Centre, IHCP/ECVAM, Ispra, Italy; [2]Doerenkamp-Zbinden Chair for alternative in vitro methods, Faculty of Natural Sciences and Mathematics, University of Konstanz, Germany

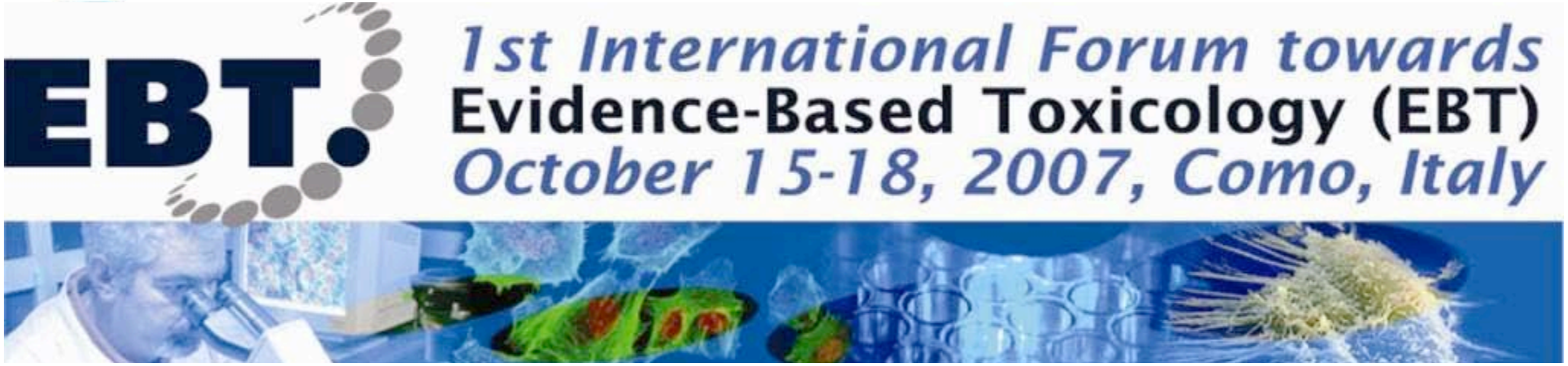
## *The evolution of toxicology*




Systems toxicology

JOHNS HOPKINS BLOOMBERG SCHOOL of PUBLIC HEALTH

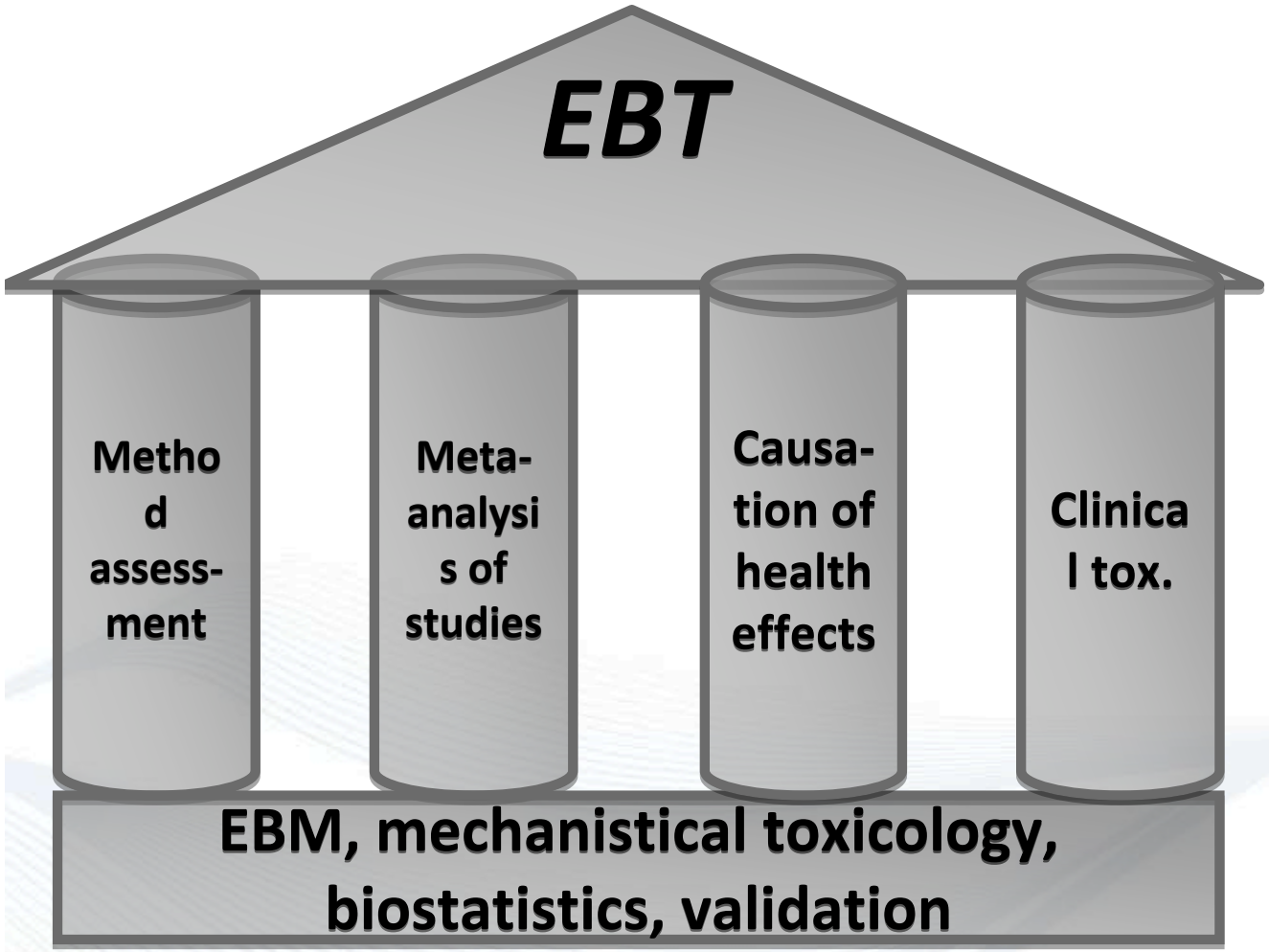

- Since 1974: „The Oxford Database of Perinatal Trials“ (3500 trials; 600 reviews)
- First Cochrane Center in 1992: Oxford, UK
- Cochrane Collaboration founded in 1993
- Today: a world-wide network of about 27.000 scientists, physicians, ... About 5.000 reviews
- US Cochrane Center at Johns Hopkins




# *Evidence-based Toxicology*

# "Evidence-based medicine goes toxicology!"

**Hoffmann and Hartung "Toward an evidence-based toxicology", Human Exp. Tox., 2006**




## What we lack:

- Data
- Information portal
- Meta-analysis & WoE tools
- Quality scoring tools
- Probabilistic risk assessment

# Food for Thought ... on Evidence-Based Toxicology

*Thomas Hartung*

Johns Hopkins University, Bloomberg School of Public Health, Dept. Environmental Health Sciences, Doerenkamp-Zbinden-Chair for Evidence-based Toxicology, Center for Alternatives to Animal Testing, Baltimore, USA

BLOOMBERG SCHOOL of PUBLIC HEALTH

# *Assessment tool for the quality of toxicological data*

- **Categorizes quality according to Klimisch scores**
- **Independent, but largely similar tools for in vivo and in vitro data/studies**
- **Expert advisory group**
- **2 rater experiments:**
  **11 rater are applying the draft tool to 11 in vitro and in vivo studies**
- **Tool now available on the ECVAM website**
- **published  Schneider et al.**
  **Tox Letters 2009, 189:138-144**
- **Impact for existing data for REACH**

Johns Hopkins University Center for Alternatives to Animal Testing


# EBT Collaboration

## Thursday, March 10, 2011

## Washington DC Convention Center

**Steering Committee (and their organizations for identification)**

**Melvin Andersen,** The Hamner Institute
**Richard Becker,** Am. Chemical Council
**Kim Boekelheide,** Brown University
**Robert Chapin,** Pfizer
**Rodger Curren,** IIVS
**Suzanne Fitzpatrick,** US FDA
**Jack Fowle,** US EPA
**Alan Goldberg,** JHU CAAT
**Thomas Hartung,** JHU CAAT
**Michael Holsapple**, ILSI/HESI
**Wendolyn Jones,** CropLife America
**Richard Judson,** US EPA
**Fran Kruszewski,** American Cleaning Institute
**Martin Stephens,** Humane Society of the US
**Bill Stokes**, National Toxicology Program
**Raymond Tice,** National Toxicology Program
**Mark Vossenaar**, Agilent
**Neil Wilcox,** FDA
**Joanne Zurlo,** JHU CAAT

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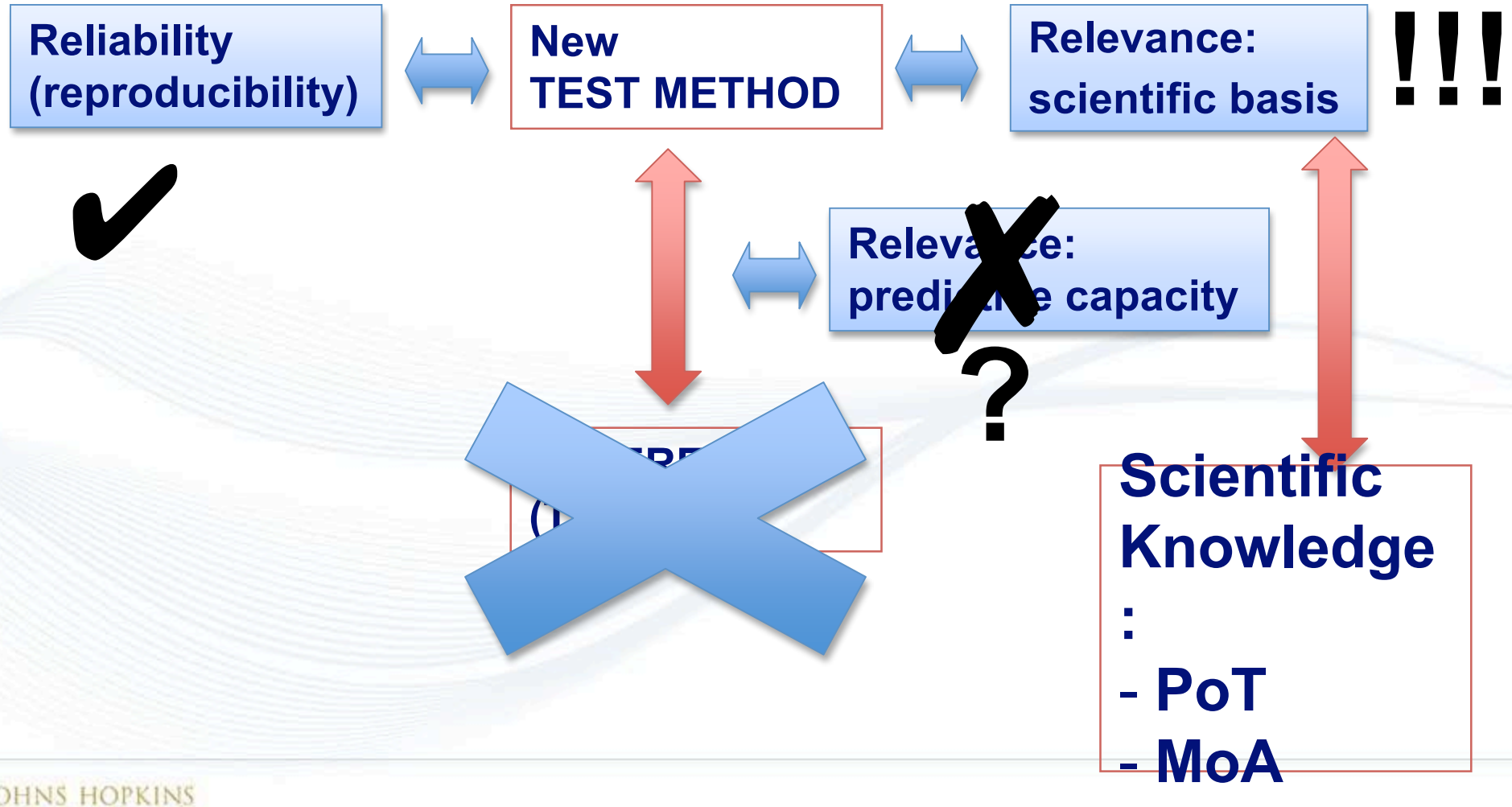
# *Definition of Validation*



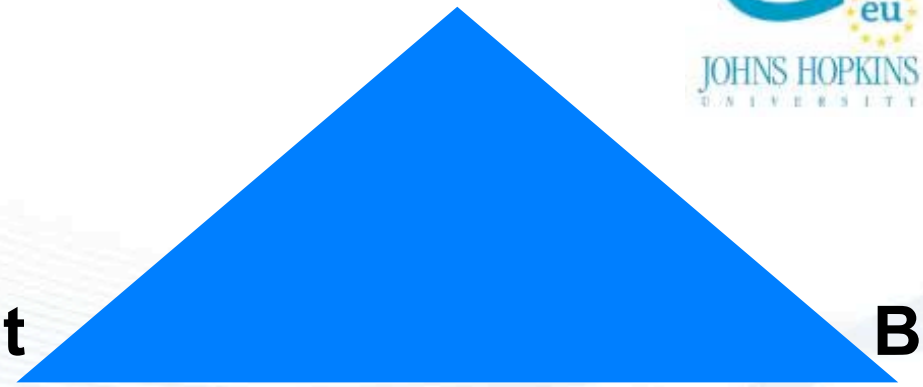
| | **Traditional validation** |
|---|---|
| **Data base** | - Prospective studies |
| **Point of reference** | - Animal test result |
| **Assessment parameters** | - Reproducibility<br>- Transferability<br>- Reliability (to predict animal) |
| **Process owners** | - Validation Management Group<br>- Trial centers |
| **Style** | - Actual testing<br>- Compilation of dossier<br>- Narrative |
| **Peer-review** | - Final dossier |
| **Publication** | - Validity statement - Scientific article<br>- evtl. Background Review Document |

**The challenge to Tox-21c will be to steer toward quality control without the creation of obstacles by formal validation. A balance between precaution and innovation is necessary, and this requires informed decisions by the actors in the regulatory arena. EBM has shown how the informed decision process in clinical medicine can be served. EBT promises to be its translation for an informed decision process in risk assessment.**

# Johns Hopkins is the right environment for EBT

# t⁴ - The Transatlantic Think-Tank of Toxicology

Konstanz



Utrecht

Baltimore

- Systematic reviews (evidence-based tox.)
- Cost-benefit analyses
- Workshops (reports)

ALTEX
AltWeb


***Nothing is as powerful as an idea whose time has come.***

**Victor Hugo**