

WORKSHOP
INCENERIMENTO E QUALITÀ
DELL 'ARIA



Sala Rappresentanza
Municipio di Bolzano
Vicolo Gumer, Bolzano
24 Ottobre 2011



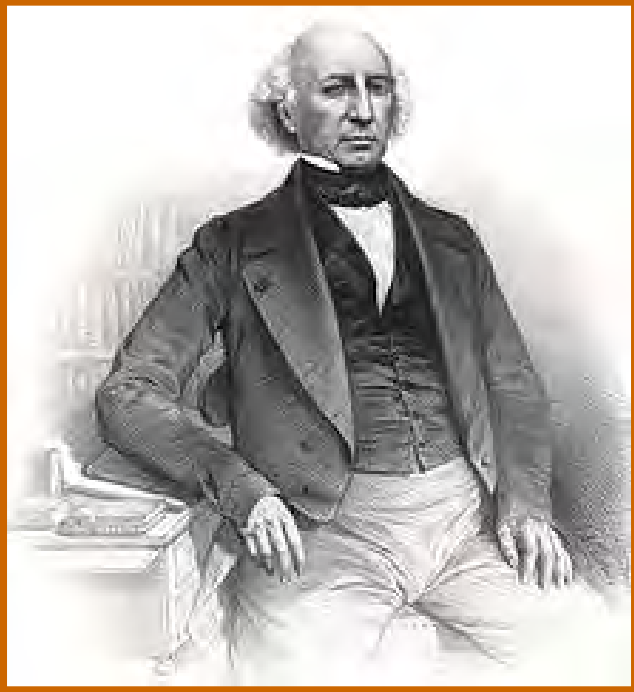
Enviromental Toxicology
in the 21st century

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Toxicology



Mathieu Orfila (1787–1853) is considered to be the modern father of toxicology, having given the subject its first formal treatment in 1813 in his *Traité des poisons*, also called *Toxicologie générale*

Toxicology (from the Greek words *τοξικός* - *toxicos* "poisonous" and *logos*) is a branch of **biology**, **chemistry**, and **medicine** concerned with the study of the adverse effects of **chemicals** on living organisms (*Schrager T, 2006*) It is the study of symptoms, mechanisms, treatments and detection of **poisoning**, especially the poisoning of people



The evolution of toxicology: patchwork

- Every scandal gives one patch
- Many patches are 50-80 years old
- No way to remove a patch
- Difficult to integrate new technologies
- Every patch is of its own appearance and workmanship

The Three Rs' of Russell & Burch (1959)

The Three Rs' concept embraces:



Rex Burch & Bill Russell
Sheringham, June 1995

- **reduction** as a means of lowering “the number of animals used to obtain information of a given amount and precision”,
- **refinement** as any development leading to a “decrease in the incidence or severity of procedures applied to those animals which have to be used”, and
- **replacement** as “any scientific method employing non-sentient material which may replace methods which use conscious living vertebrates”

Traditional toxicological tests

- **Based largely on the use of laboratory animals**
“toxicology has survived with essentially one test per hazard”
- **This approach suffers from**
 - **low throughput**
 - **high cost**
 - **difficulties inherent to inter-species extrapolation**
 - ⇒ each test can reflect the human situation only in part
 - ⇒ grey zone → differences in various subpopulations, use scenarios, and cofactors

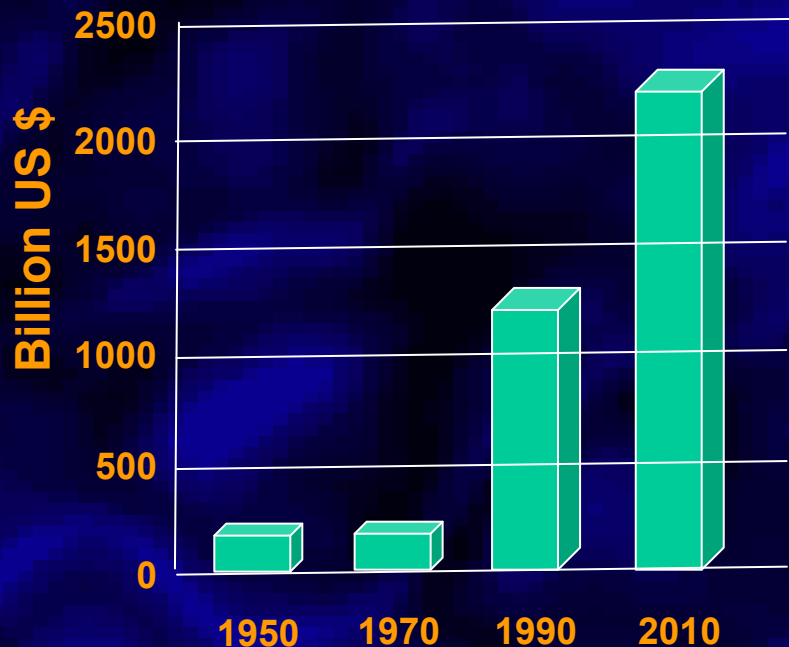


Toxicology
desperately needs
to renew its
toolbox

In fact, this approach is of limited use in evaluating the very large number of chemicals with inadequate toxicological data

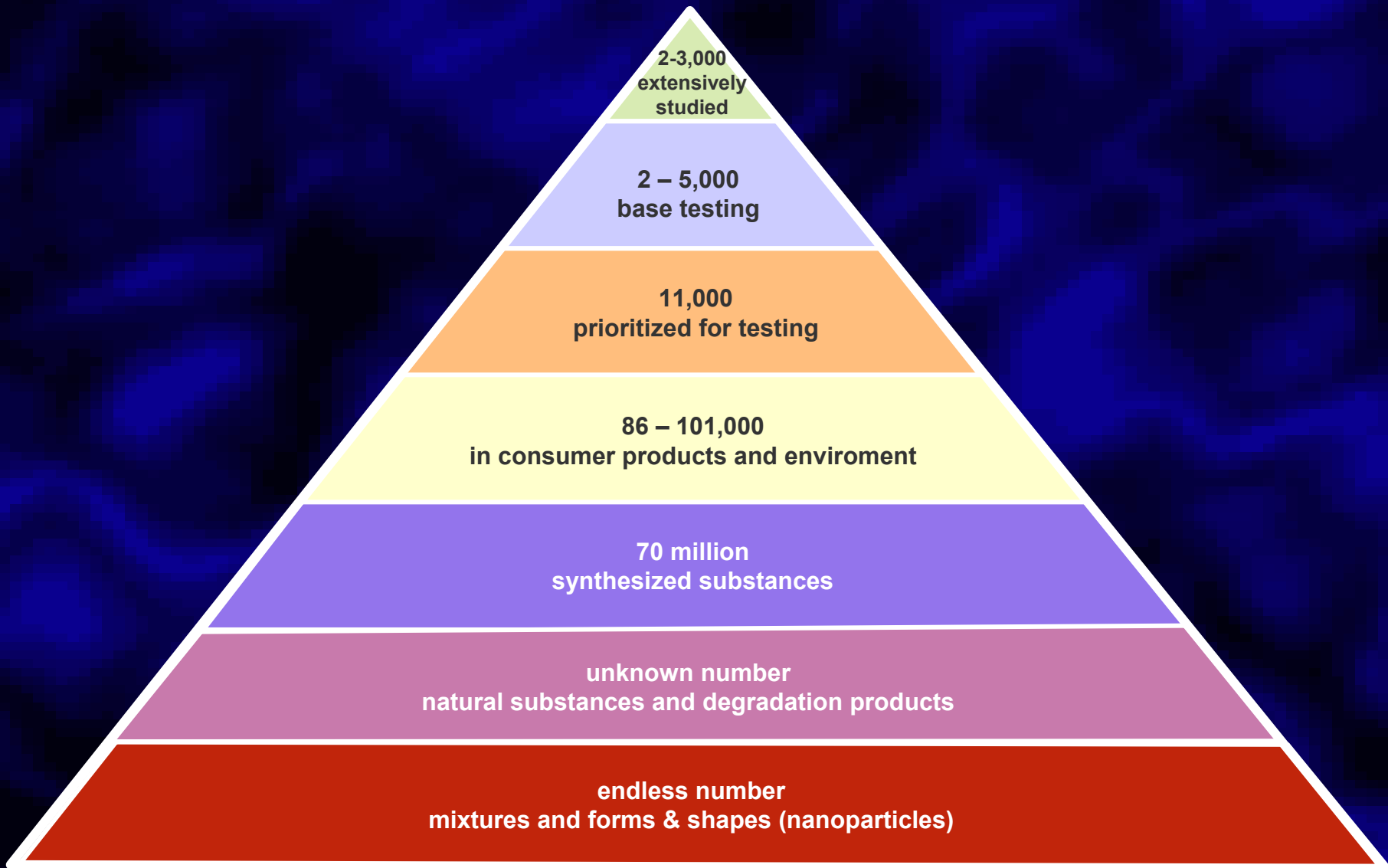
Large Numbers and Volumes of Chemicals are Produced

World Chemical Production



- Chemical production has increased spectacularly since 1970s
 - Expansion of chemical portfolio
 - Expansion of types of products
 - Ubiquitous integration
- Formidable number of chemicals in commercial use
 - 143,000 substances in Europe
 - 100,000 in US
- Approximately 30,000 substances marketed in volumes > 1 t/y
 - About 3,000 High Production Volume chemicals make up 95% of total production

The chemical universe of toxicological knowledge



Formation of the European Union (EU) and Legislative Actions That Stimulated the Development of Alternative Methods

- **Treaties Rome (1957/58)**
 - European Economic Communities (EEC)
- **Cosmetics Directive (1978)**
 - Directive 76/768/EEC
 - Main European law on the safety of cosmetics
- **Maastricht Treaty (1992/93)**
 - Formation of the European Union (EU)
 - European Commission (EC)

Significant Changes in the European Union Have “Jump Started” the Development of Alternative Methods

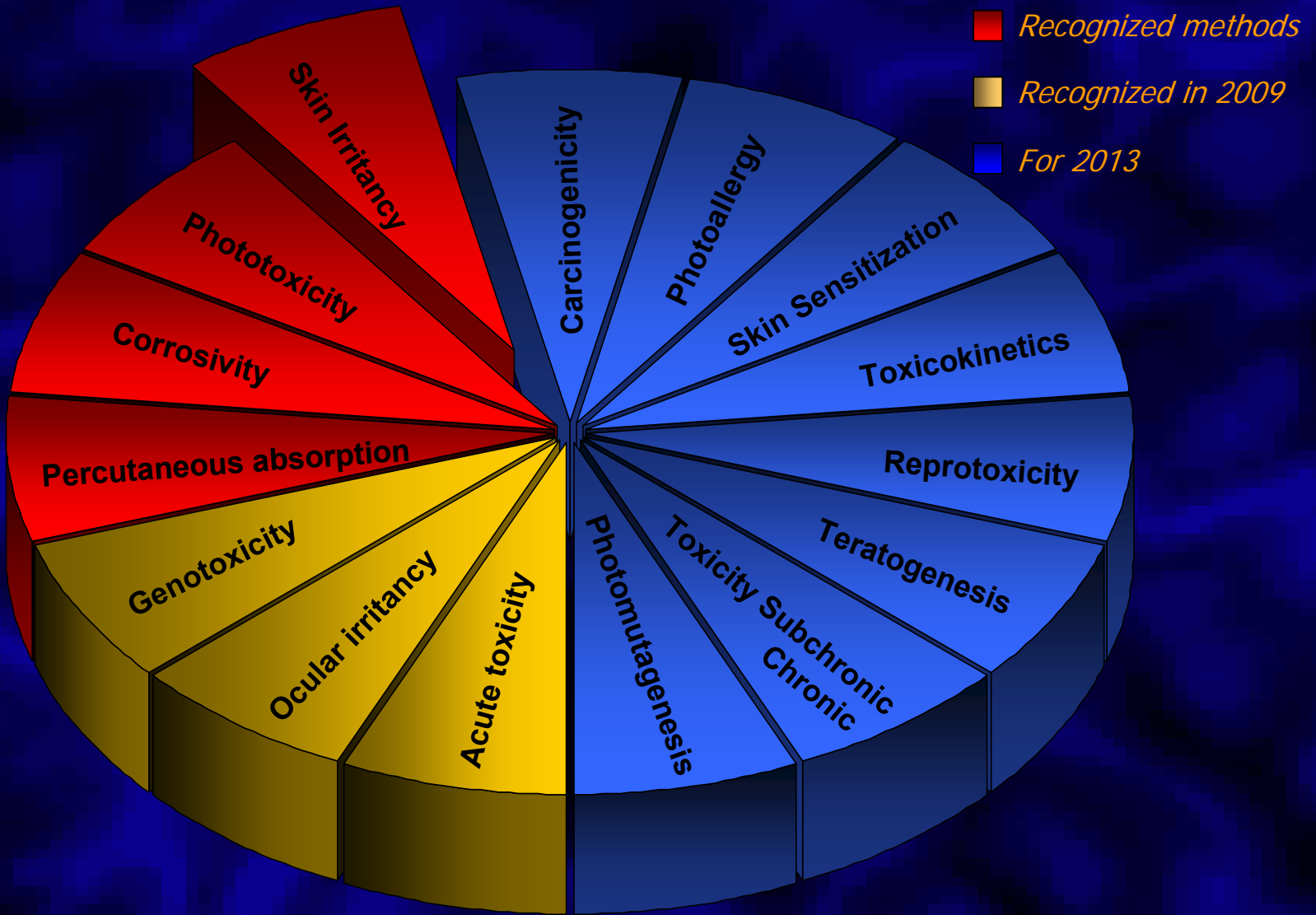
- **Amendment/Annex VII**

Today's the Day: Animal Testing Ban Initiated in Europe

Posted: March 11, 2009

- Bans the use of animals for evaluating the safety of cosmetics
- Bans the sale of cosmetics if ingredients were tested on animals

Implementation and Timing of Amendment VII



The REACH Initiative Could Cost Billions of Dollars and Use Millions of Animals

- **Registration, Evaluation, and Authorization of Chemicals (REACH)**
 - Industry is responsible for chemical risk (production, transport, labeling)
 - Relevant toxicology:

Safety Testing Required by REACH

1 to 10 Tons/year

- Physical and chemical
- Skin corrosion
- Skin irritation
- Eye irritation
- Skin sensitization
- Mutagenicity
- Acute oral toxicity

> 10 Tons/year

- Skin irritation
- Eye irritation
- Mutagenicity
- Acute toxicity
- Repeat dose toxicity
- Reproductive toxicity

Thousands of chemicals
Require millions of animals
billions of dollars

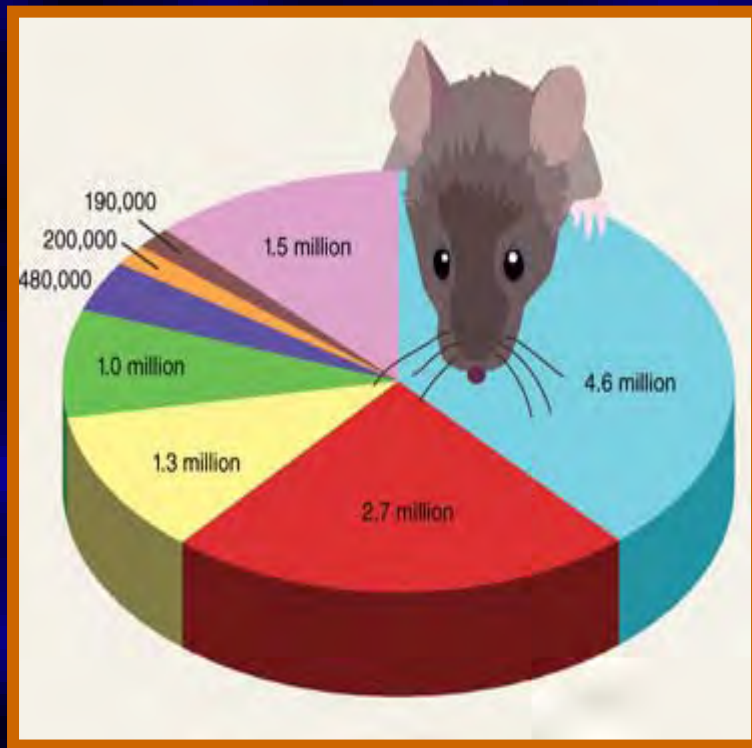
In Vitro methods welcomed

Does REACH Allow for *In Vitro* Data?

- *Article 13: General requirements for generation of information on intrinsic properties of substances*
- **In particular for human toxicity, information shall be generated whenever possible by means other than vertebrate animal tests, through the use of alternative methods**, for example, *in vitro* methods or qualitative or quantitative structure-activity relationship models or from information from structurally related substances (grouping or read-across)

Number of animals used for scientific purposes in the EU's 27 in 2008

Total number **12 million**

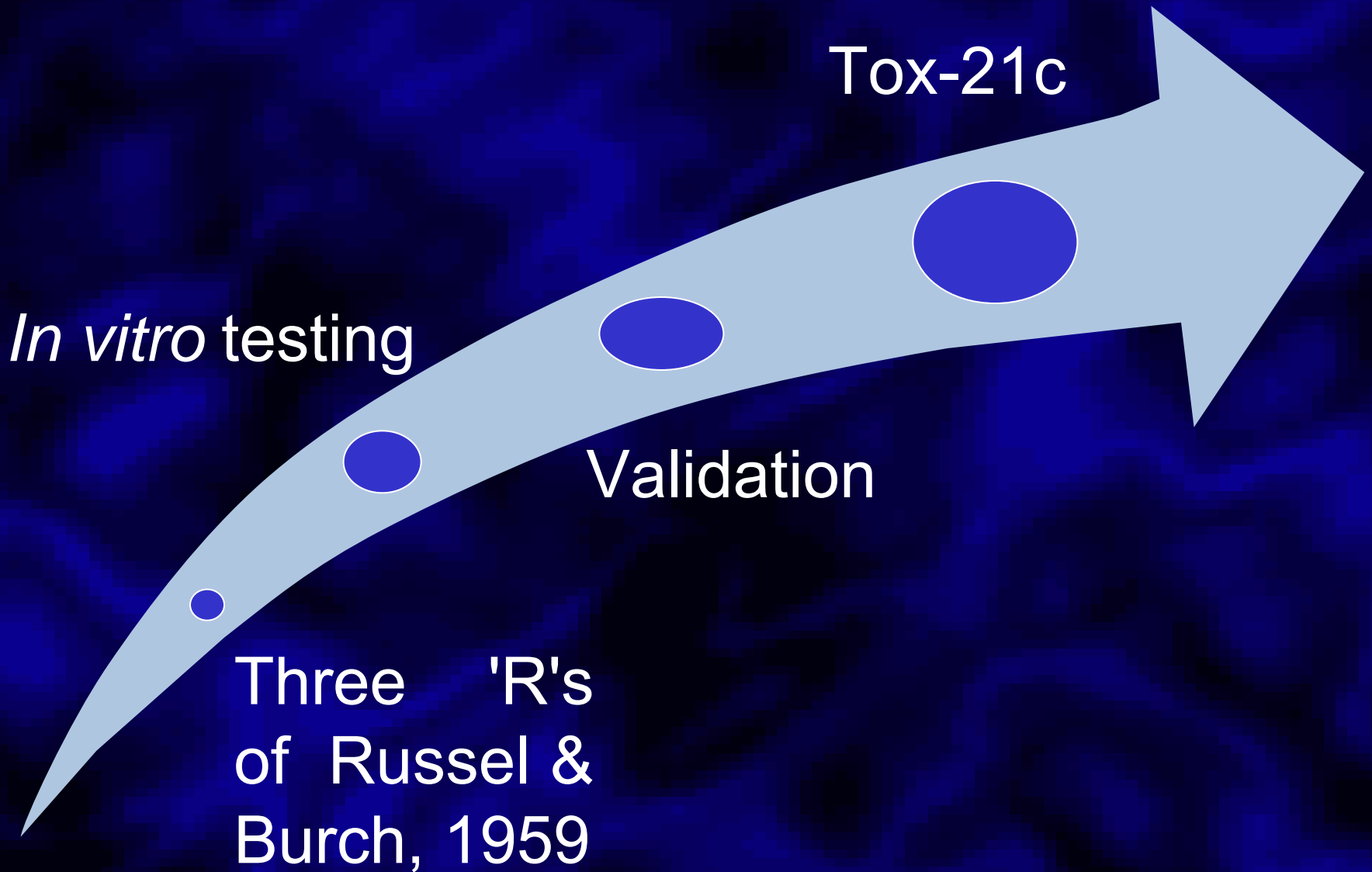


- Fundamental biology studies (39.1%; 4.1%)
- Research & development (22%; -8.2%)
(human, veterinary, dentistry)
- Production & quality control (10.6%; 4.5%)
(human medicine, dentistry)
- Toxicological & other safety evaluation (8.1%; 0.4%)
- Production & quality control (3.9%; 0.5%)
(veterinary medicine)
- Education & training (1.6%; 0%)
- Diagnosis of disease (1.5%; -0.1%)
- Others (13%; -1%)

Percentage and fluctuation compared to 2005 in brackets

Dolgin, Nature med., 16(1172), 2010

Toward humane science



What is "Tox21"?

Is a collaboration between

- the National Institute of Environmental Health Sciences/National Toxicology Program
- the National Institutes of Health/National Chemical Genomics Center (NCGC)
- the Environmental Protection Agency (EPA)
- and most recently the Food and Drug Administration (FDA)

The Value of In Vitro Data For Assessing Drug and Chemical Safety Issues is Now Recognized as a Viable Approach

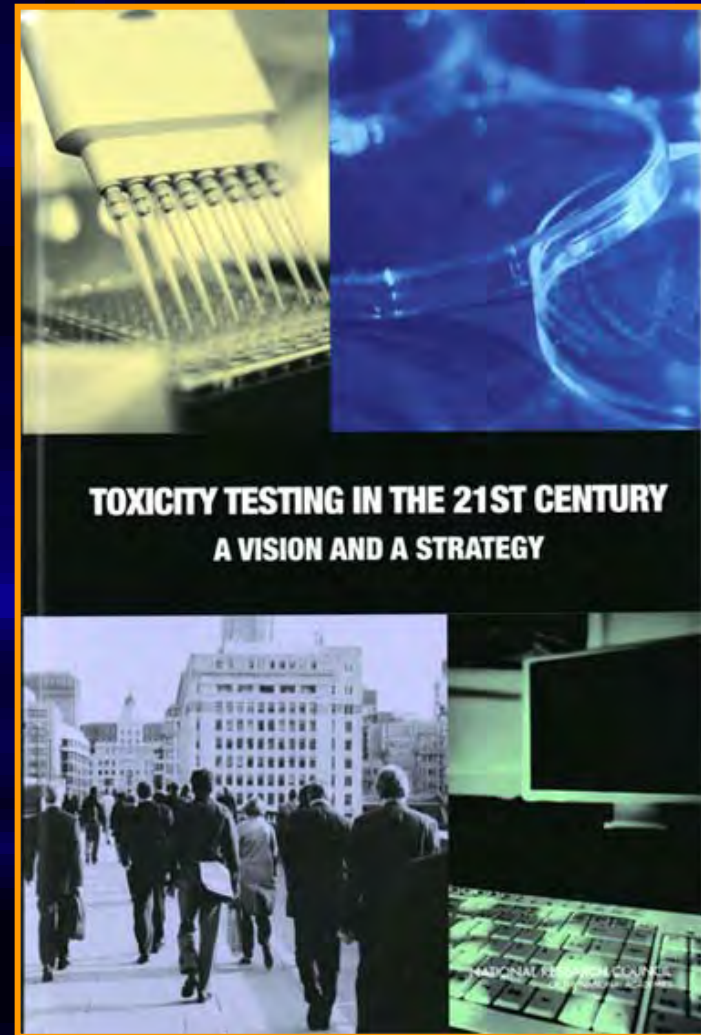
National Academic of Science Report
*Toxicity Testing in the 21st Century:
A Vision and Strategy, 2007*

Animal studies: time consuming and expensive

Lack of predictability of animal tests

Use of human cells in culture

Systems biology and pathways = mechanisms



**TOXICITY TESTING IN THE 21ST CENTURY
A VISION AND A STRATEGY**

US Environmental Protection Agency Launched ToxCast in 2007

The ToxCast Program for Prioritizing Toxicity Testing of Environmental Chemicals

David J. Dix, Keith A. Houck, Matthew T. Martin, Ann M. Richard, R. Woodrow Setzer and Robert J. Kavlock

Computational Chemistry
High Throughput Screening
Toxicogenomic Technologies

Predict the potential for toxicity
Prioritize chemicals for animal testing



Both Europe, The United States, and Japan, and Have Agreed to Support Development of *In Vitro* Methods

- European Commission: ECVAM – European Center for the Validation of Alternative Methods
- Japanese Center for Validation of Alternative Methods: JaCVAM
- US National Institutes of Environmental Health Sciences (NIEHS): National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)
- ICCVAM – Interagency Coordinating Committee on the Validation of Alternative Methods
- Organization for Economic Co-operation and Development (OECD) – Global Harmonization and Oversight

There is only one validation process



**Collaboration with
ECVAM, JaCVAM,
KoCVAM also in
ICATM**

**OECD guidance
document 34**

**... but difference in details, e.g. resources, active validation,
peer-review, acceptance**

The new frontiers of toxicological tests

- The dramatic technological advances in molecular biology and computer science
⇒ opportunity to **use *in vitro* biochemical- and cell-based assays and non-rodent animal models** for toxicological testing
- These assays allow for much higher throughput at a much reduced cost
⇒ many thousands of chemicals can be **tested simultaneously** in days

The new frontiers of toxicological tests

- The goal is to move toxicology
 - ⇒ from predominantly **observational science** at the level of disease-specific models
 - ⇒ to predominantly **predictive science** focused upon a broad inclusion of target-specific, mechanism-based, biological observations

High Throughput Screening (HTS) program

- The HTS program approach to toxicological testing **screens for mechanistic targets active within cellular pathways considered critical to adverse health effects** such as carcinogenicity, reproductive and developmental toxicity, genotoxicity, neurotoxicity, and immunotoxicity in humans

Goal of HTS program

- To prioritize substances for further in-depth toxicological evaluation
- To identify mechanisms of action for further investigation (e.g., disease-associated pathways)
- To develop predictive models for *in vivo* biological response (predictive toxicology)

The Emerging solutions

- **Apply the new technologies**
- **Integrated testing strategies**
- **Pathways of Toxicology (PoT)**
- **Probabilistic risk assessment**
- **Evidence based toxicology (EBT)**

The Emerging solutions

- **New technologies**



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.**”

The Emerging solutions

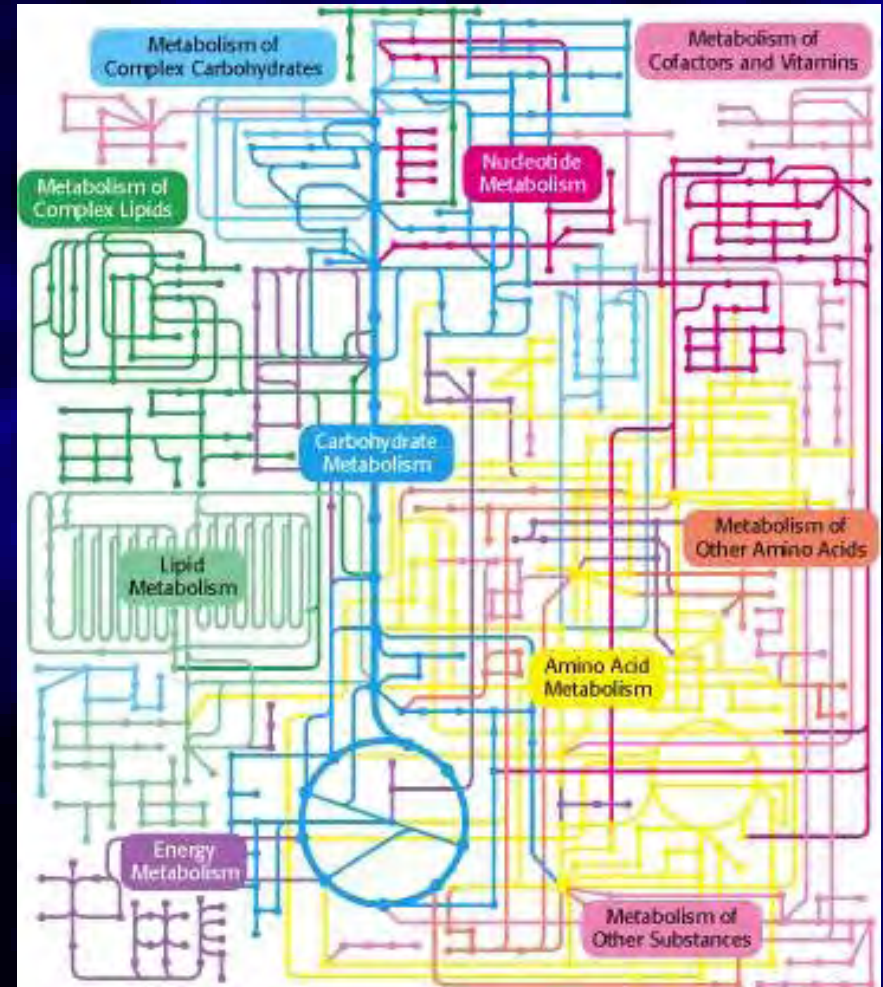
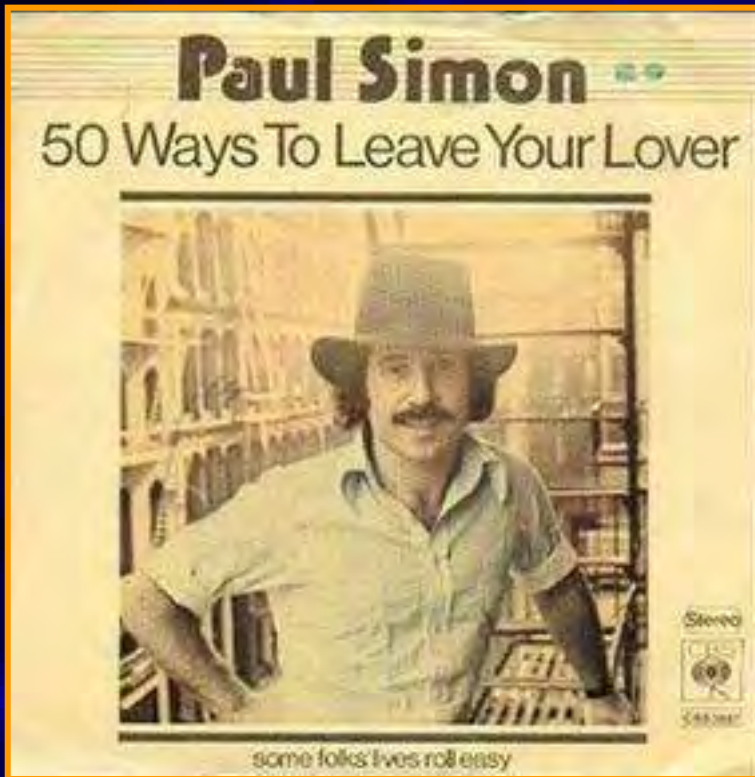
- **Integrated testing strategies**

- A test can be optimized to be either **sensitive** (*few things missed*) or **specific** (*few false accusations*)
- We have to give up the illusion of “**definitive test**” → it is unrealistic that one test covers all modes of action
- However, to date, we do not have strategies, knowledge of how to compose, validate, or adapt them
- We also **lack routine statistics** for such complex decision trees. We might learn from clinical diagnostics, where similarly tests are combined for differential diagnosis. In many areas, decision theory has flourished, but in safety assessment of chemicals there are enormous opportunities

The Emerging solutions

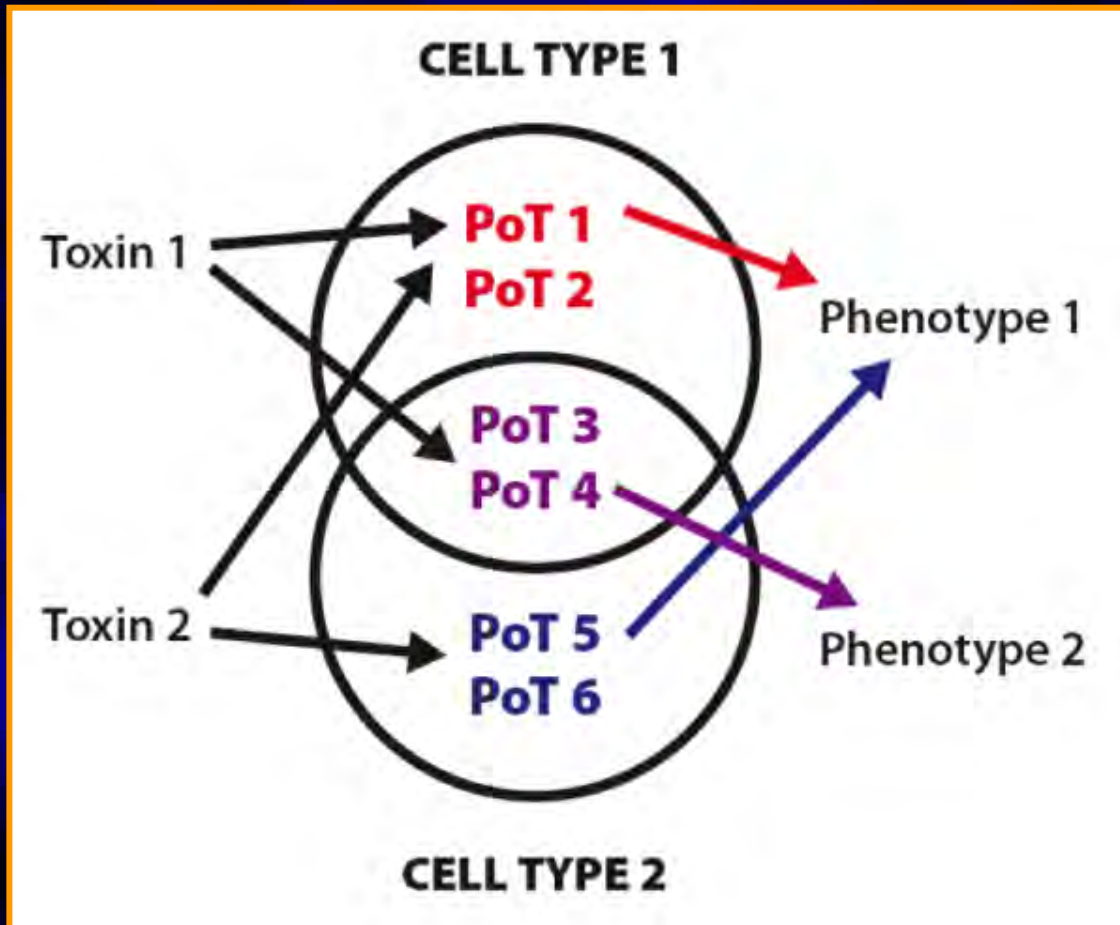
- **Pathways of Toxicology (PoT)**

The central idea of Tox-21c is that a finite number of distinct PoT exist→ if we are able to map them, we can build a test battery to cover them



...and a couple of hundred ways to kill a cell

Mapping the (finite number of) pathways of toxicity



Annotation to:

- Cell type
- Species
- Hazard
- Toxin (class)

The Emerging solutions

- Probabilistic risk assessment

A substance is a carcinogen or not

Vs.

It has a certain probability to induce cancer in a given use scenario



Our tests cannot determine carcinogens but estimate this probability with some uncertainty



This is what science can actually deliver

The Emerging solutions

- **Probabilistic risk assessment**

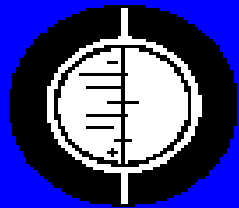
This will make life more difficult but will also make clear what a toxicological test actually does

⇒ **it changes the pre-test probability of a hazard to a post-test probability**

The Emerging solutions

- Evidence based toxicology (EBT)

Evidence based Medicine



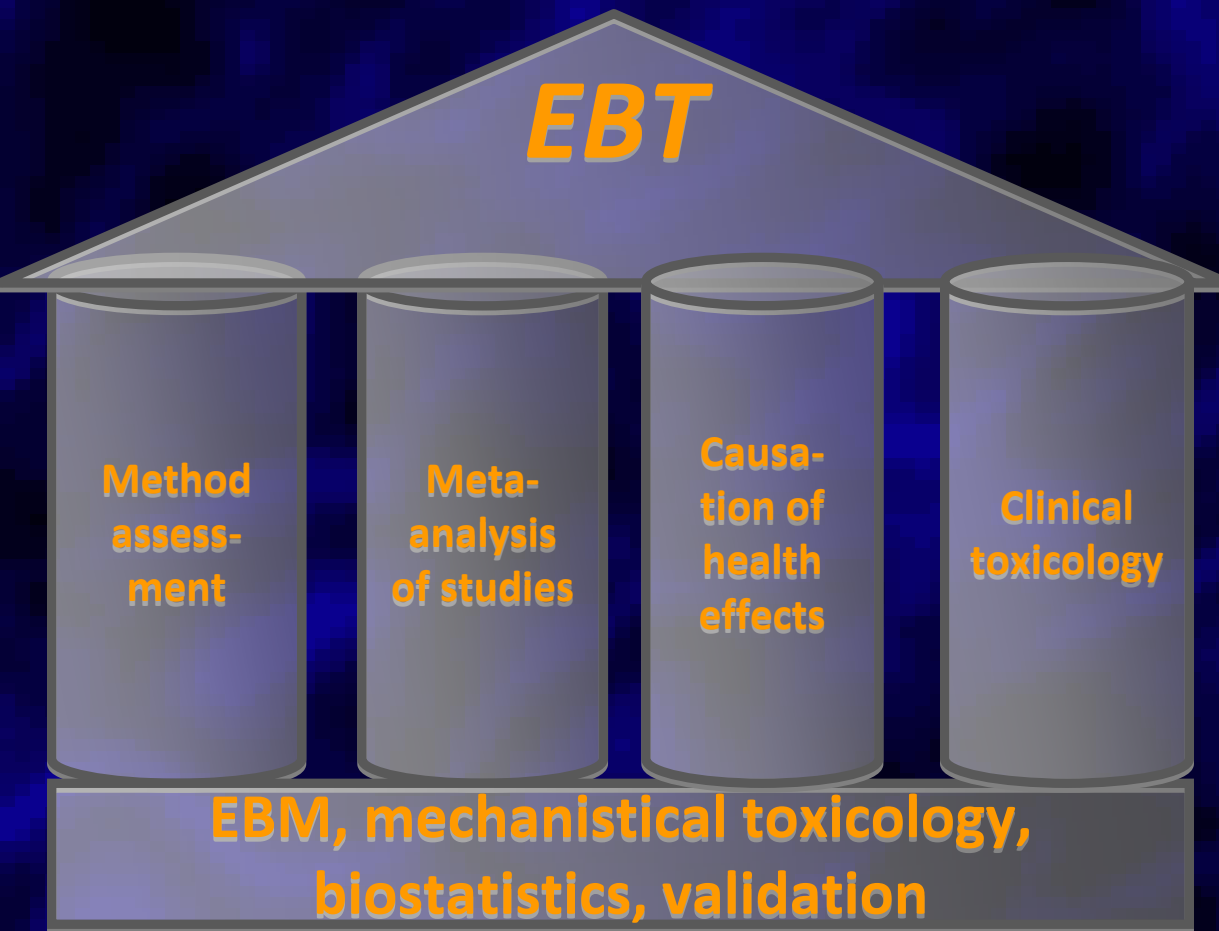
**The Cochrane
Collaboration**

Main Tools

- Defines the sources
- Weighting the quality
- Meta-analysis

- 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
- Italian Cochrane Center at Mario Negri, Milan

The Emerging solutions



What we lack:

- Data
- Information portal
- Meta-analysis & Weight of Evidence 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

The basic idea for a new toxicology

Retro physiology-base PK

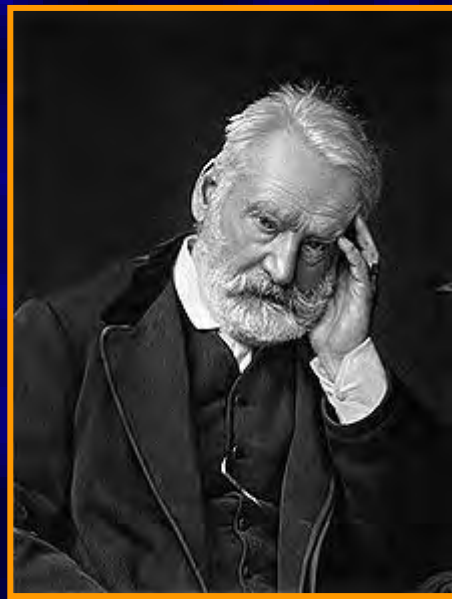
Probabilistic risk
assessment

PoT
identification

EBT

Integrate
testing
strategies





**Victor Hugo
(1802-1885)**

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