A scientific roadmap for animal-free systemic toxicity testing

Dr. Nina Hasiwa
CAAT-Europe, University of Konstanz
AtaX-Advice, Alternatives to Animal Xperiments

Who we are…

Doerenkamp-Zbinden Chair
'**Evidence-based Toxicology**'
John Hopkins University
Baltimore, US

Doerenkamp-Zbinden Chair
'**In vitro Toxicology and Biomedicine**'
University of Konstanz
Konstanz, Germany

CAAT-Europe as a joint venture

Belgium

Germany

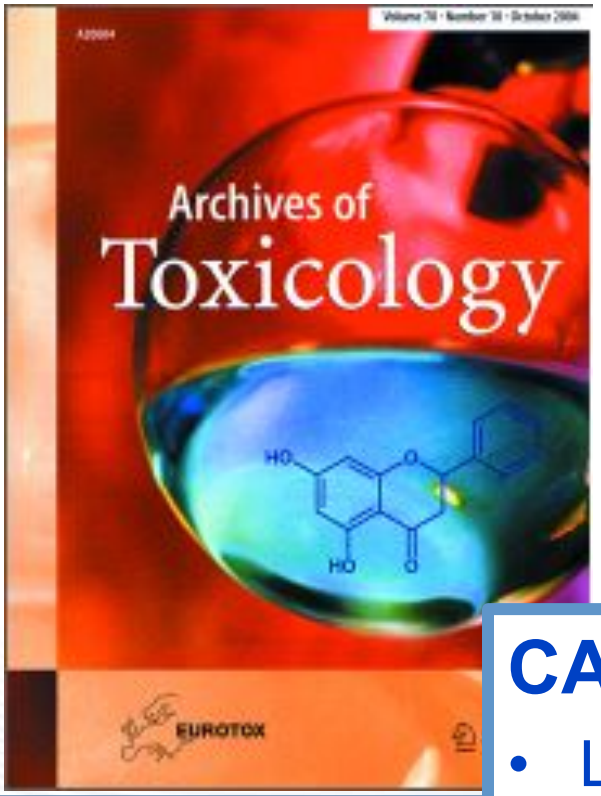
Italy





… and more than 35 experts from all over the world

EC Report, Adler 2011



2010

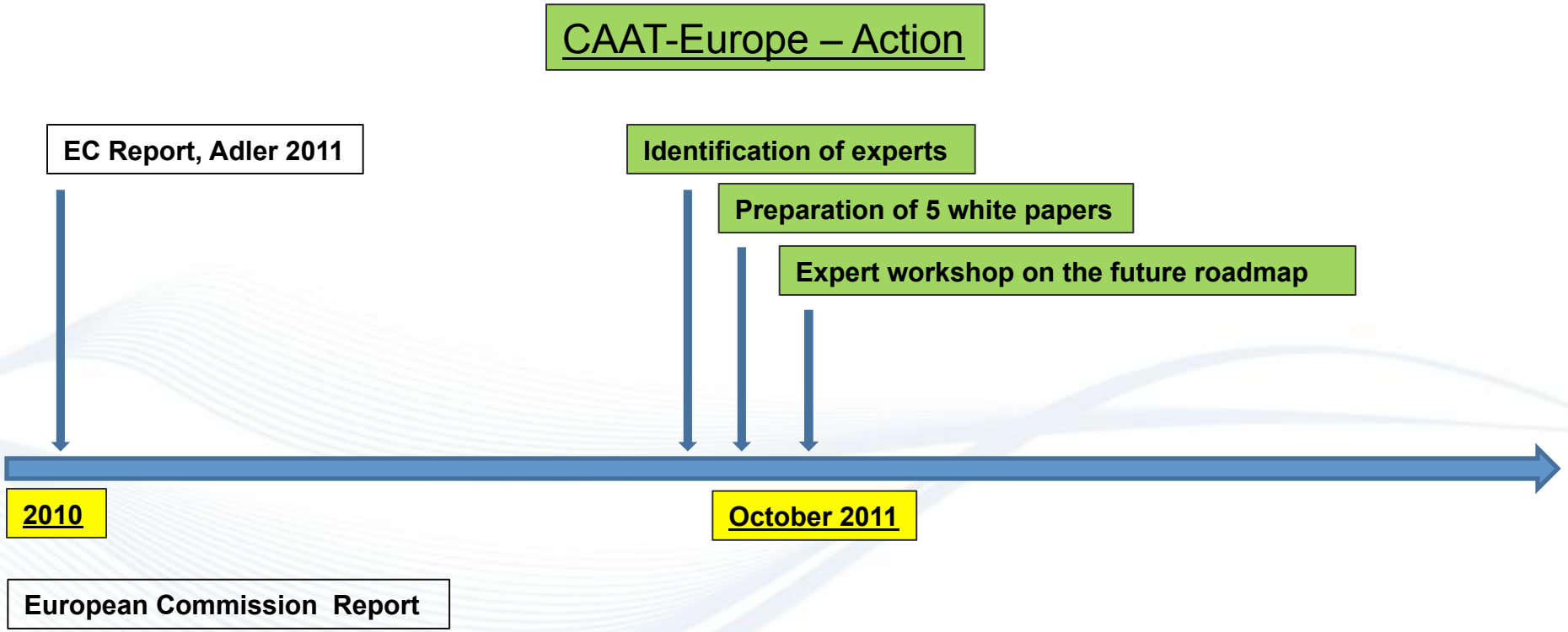
European Commission Report

CAAT's intention

- Lessons from the past?
- Ways forward?

→ Perspective for the future?

Scientific roadmap for the future of animal-free systemic toxicity testing



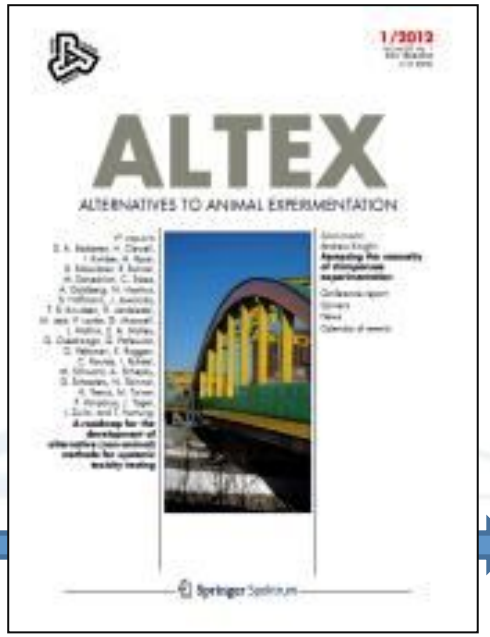
Scientific roadmap for the future of animal-free systemic toxicity testing

CAAT-Europe – Action

EC Report, Adler 2011

Expert workshop on the future roadmap

January 2012



2010

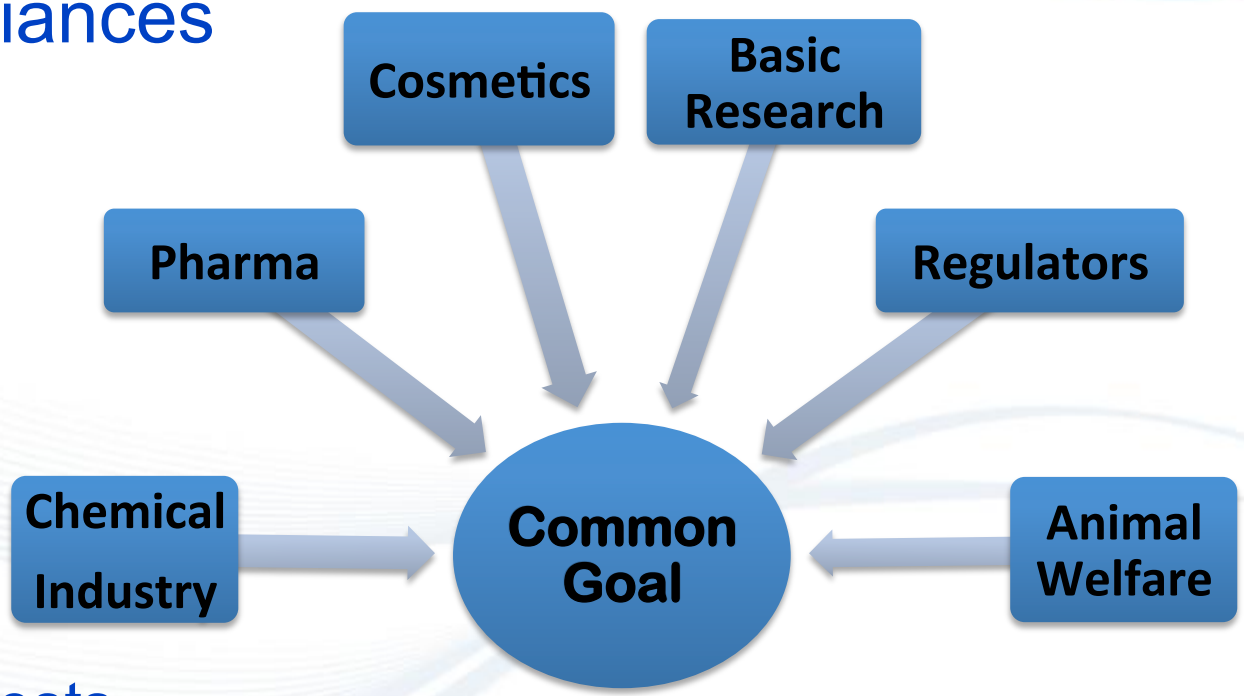
October 2011

European Commission Report

Publication of the roadmap in ALTEX

General Recommendations

- **Join Forces**
  - New alliances

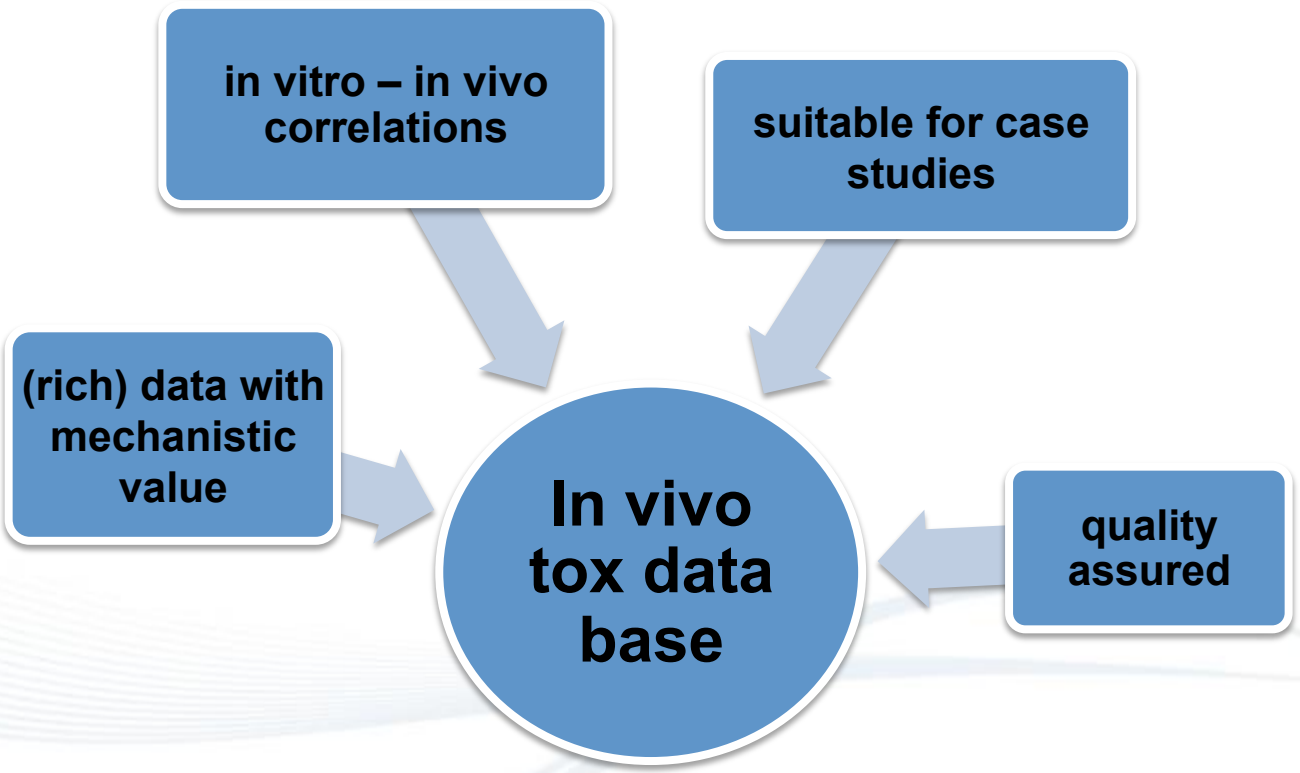


→Shared interests
→Common projects
→Shared data

→ In vivo tox database

Already existing

- OpenTox
- EPA
- IMI activities



Major hurdles

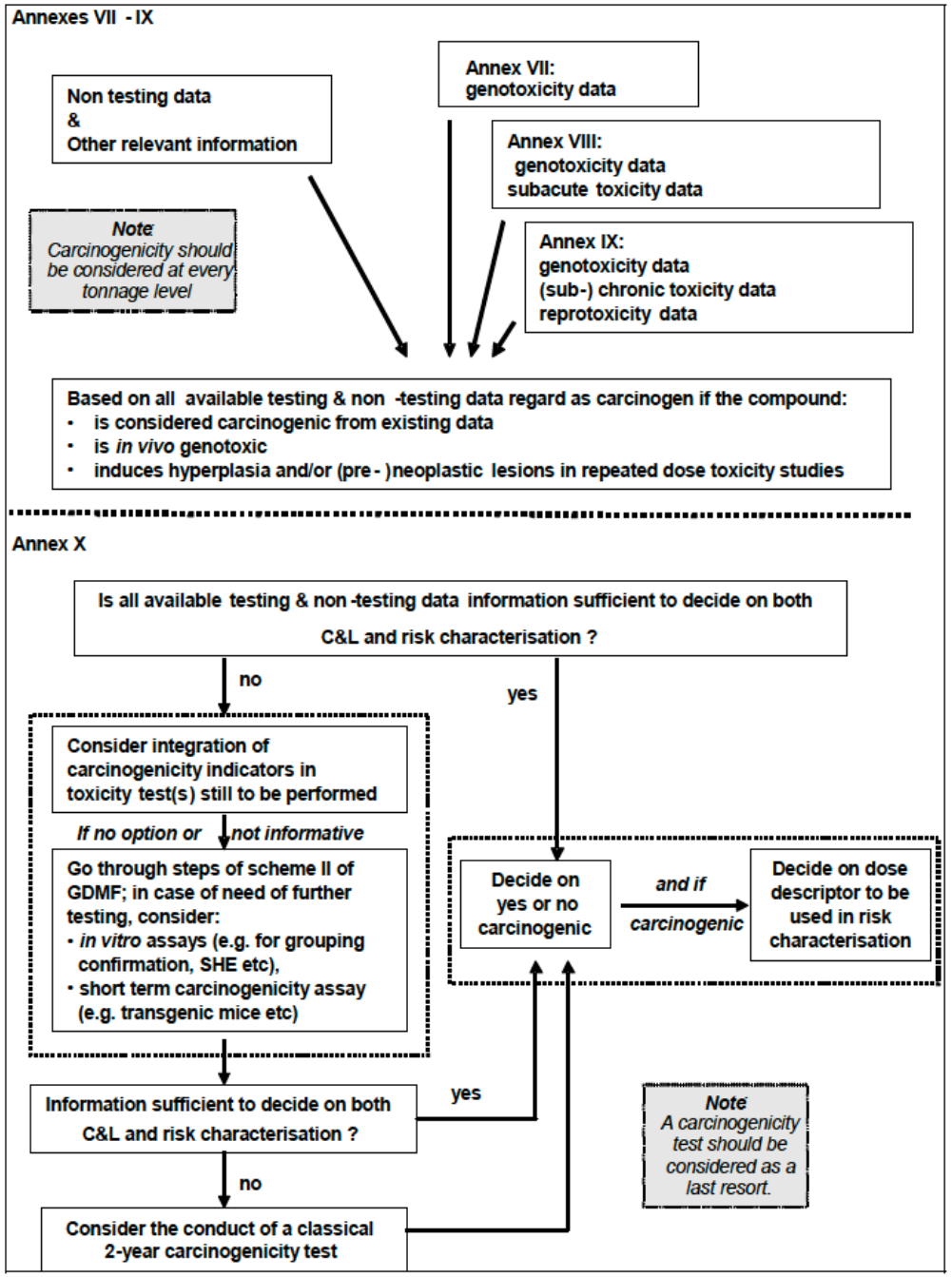
Huge hesitations in sharing data

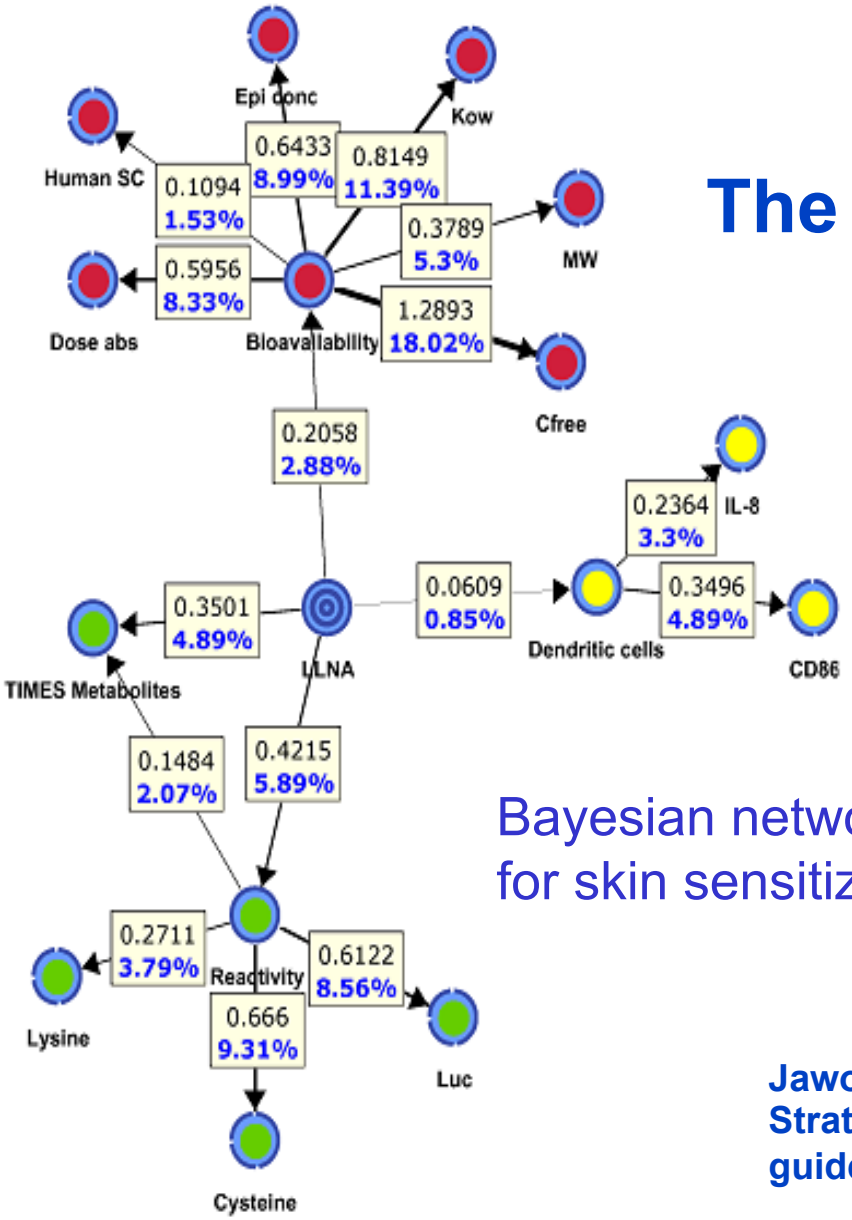
Big effort in organization and co-ordination

General Recommendations

- Join Forces
- **Integrated testing strategies**

Figure R.7.7-2 Integrated Testing Strategy for carcinogenicity





The future of ITS

- **Probabilistic / adaptive**
- **Machine Learning**
- **Interim decision points**

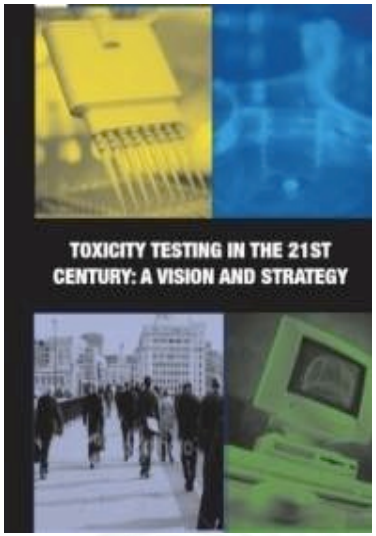
Bayesian network ITS
for skin sensitization

Jaworska, J., and S. Hoffmann. 2010. Integrated Testing Strategy (ITS) - Opportunities to better use existing data and guide future testing in toxicology. *ALTEX* 27: 231–242.

General Recommendations

- Join Forces
- **Integrated testing strategies**
- **Computer-based models**
  - Focus on ITS
  - Control of input (learning sets)
  - Start now, involve regulators early
- **Pathways of Toxicity (PoT)**

Pathways of Toxicity



Exposure

⬇

Tissue Dose

⬇

Biologic Interaction

⬇

Perturbation

Higher

Biologic Inputs

Normal Biologic Function

Early Cellular Changes

Adaptive Stress Responses

Cell Injury

Morbidity and Mortality

http://www.nap.edu/catalog/11970.html

Thomas Hartung, presentation Cancer

Emerging Initiatives

Organization	Approach	Purpose	Outcome
US EPA (Toxcast Program)	High-throughput testing	Chemical prioritization (initially)	"Biological signatures"
Hamner Institutes	Case studies	"Just do it"	Proof-of-principle
CAAT-US	Pathway mapping	Pathway ID & annotation	Human Toxome

General Recommendations

- Join Forces
- Integrated testing strategies
- Computer-based models → in silico
- **Pathways of Toxicity (PoT)**
  - Annotation to cell types
  - Physiological context

General Recommendations

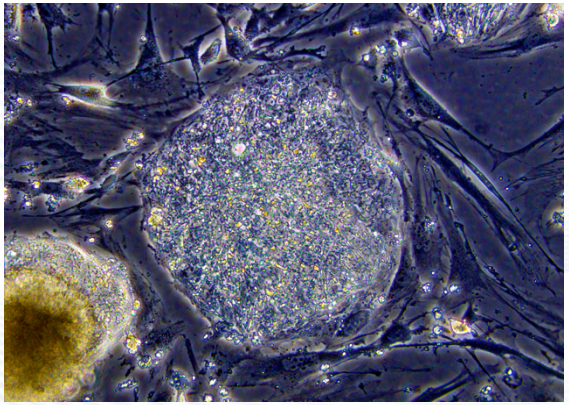
- Join Forces
- Integrated testing strategies
- Computer-based models → in silico
- Pathways of Toxicity (PoT)
- **In vitro methods**
- **Optimization of existing test systems**

In vitro methods

Humanized test systems, Stem cells

Multiple endpoints (functional, organ-specific)

Combination of simple and complex methods

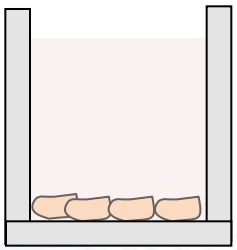


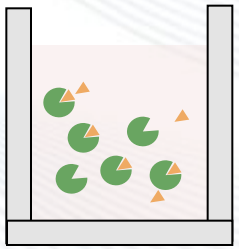
Trosko 2004

General Recommendations

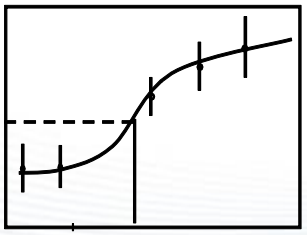
- Join Forces
- Integrated testing strategies
- Computer-based models → in silico
- Pathways of Toxicity (PoT)
- In vitro methods
- Optimization of existing test systems
- **Biokinetics**

In the future: Biokinetics is **necessary** to relate the nominal concentration in an *in vitro* assay to the equivalent *in vivo* human exposure





In Vitro Toxicity Assays



EC_{50} or Data on Concentration-Response

QIVIVE*

Estimate of Human Equivalent Dose

* **Quantitative *In Vitro* to *In Vivo* Extrapolation**

Harvey Clewell, presentation TK

General Recommendations

- Join Forces
- Integrated testing strategies
- Computer-based models → in silico
- Pathways of Toxicity (PoT)
- In vitro methods
- Optimization of existing test systems
- QUIVIVE
- **Abolition of useless tests**

Abolition of useless tests

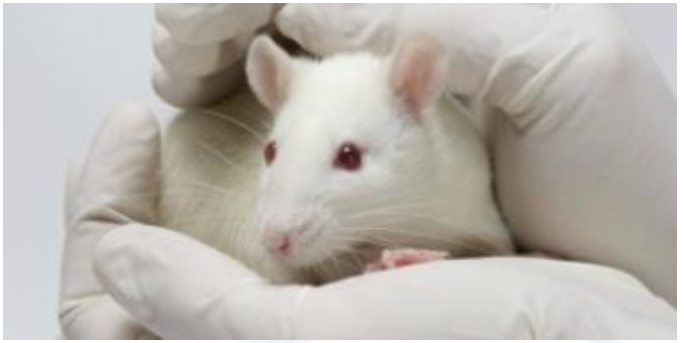
Cancer Bioassay

18-24 months

$1-1.5 million

600 animals

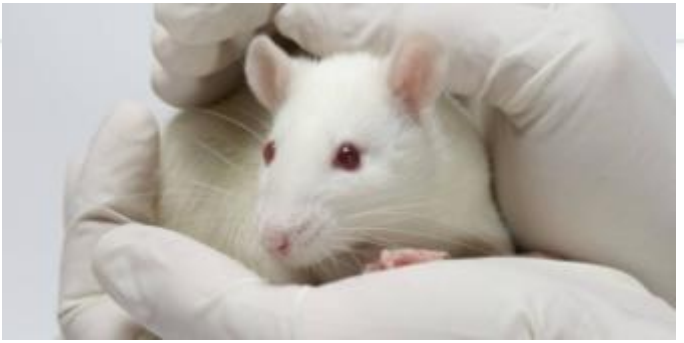
53% positive*



Estimate human 5-20% positive

*Ames&Gold Mut.Res / 2000

→ **High rate of false-positives**



Validity of the Cancer bioassay

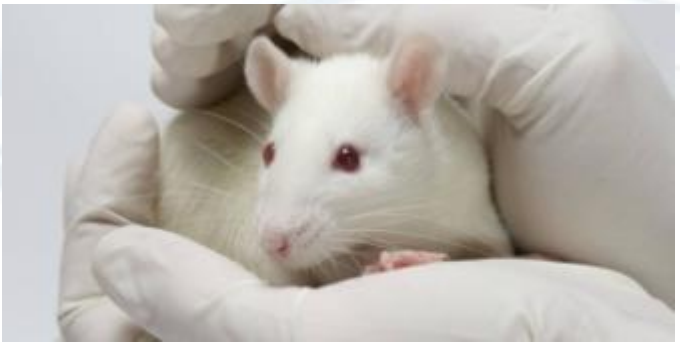
1) Not robust (13% equivocal / not adequate studies)
2) Not standardized (strain, statistics..)
3) 57% reproducibility [Gottmann 2001]
4) Mouse to rat 57% correlation
5) 69% rat to human predictivity [Pritchard 2003]
6) EPA: 58% of positive studies → no classification

Example: Risk assessments of trichloroethylene

Carcinogen	Equivocal	No carcinogen
4 studies	19 studies	6 studies

IARC human carcinogens, negative in two species cancer bioassay:

Nickel sulfate
Asbestos (oral)
Phenacetin
Magnesium silicate
Diarylamide yellow
2-Napthylamin
8-Methoxysporalen (w/o UV)



General Recommendations

- Join Forces
- Integrated testing strategies
- Pathways of Toxicity (PoT)
- In vitro methods / Optimization
- Biokinetics / QIVIVE
- Abolition of useless tests

Specific Recommendations Toxicokinetic

Characterization of **free concentration** in cell-based assays

***In vitro* models** for absorption, barriers, clearance

Development of **generic PBPK modeling platforms**

- user friendly, open access
- database for physiological parameters
- multiple parallel metabolic pathways

Data collection to support QSPR modeling

QIVIVE **case studies**

Specific Recommendations Skin Sensitization

- Reasonably good animal model (LLNA)
- Good set of in vitro assays available

→**final evaluation**

- Need of data integration in ITS

→**Join forces**

→**Multidisciplinary collaboration**

Specific Suggestions Carcinogenicity

- Evaluation of current assay
  → **abolition of current test**
- Optimize battery for genotoxic carcinogens
  → **new assays** of repair, recombination, …
- ITS including **'non-genotoxic'** modes of action
- **Further evaluation** of "CTAs"

Specific Suggestions Reproductive Toxicity

- Evaluation of current assay
  → **abolition of current test**
- Validation of (human) embryonic stem cell tests (**'ESTs'**)
- Validation zebrafish egg test (**'DART'**)
- Extension of ITS approaches **'ReProTect II'**

What to do tomorrow?

ITS: skin sensitization as learning model

Expansion on ReproTox

Quality control of current tests

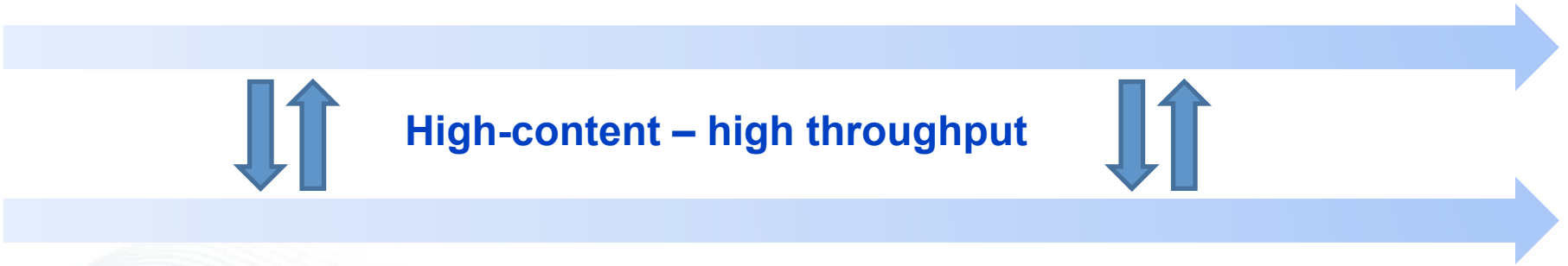
Case studies → Repeat-dose toxicity

accelerated

→ Validation
→ Acceptance
→ Implementation

The day after tomorrow??

Complex test systems: 3D or stem cell models with functional endpoints



Pathways-of-toxicity: simple pathway-based test systems

Deterministic risk classification

→ probabilistic risk assessment

- Start now
- Join forces
- Find ressources
- Think outside the box

Thanks for your attention

... animal-free systemic toxicity testing