

Replacement of Regulatory Animal Studies by Alternative Methods

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Outline

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Use of Experimental Animals

“Experimental animals
are used in development processes of
several product types as
safety and efficacy guarantee
for human health”

START-UP Final Report, 2010

- Chemicals
- Pharmaceuticals
- Agrochemicals
- Pesticides
- Food constituents

Replace Animal Experimentation

- 1959 W.M.S. Russel and R.L. Burch
„The Principles of Humane Experimental Technique“
 - ∅ Basic Principle: Optimal housing and treatment of experimental animals is prerequisite for high quality results in animal experiments
 - ∅ Key words: reduce, refine, replace
 - ∅ Final Goal: replace animal experimentation through alternative methods
- Basic dream
Replace 1 Alternative Method replaces 1 Animal Test (1:1)
- Clear from beginning
Each in vitro method is less complex than in vivo
- è Challenge: despite qualitative reduction → acceptable safety prediction
- è Solution (?): Integrated/Intelligent Testing Strategies (ITS)

http://altweb.jhsph.edu/pubs/books/humane_exp/het-toc

Central Theme - 3 R's

Replacement, Refinement and Reduction

Replacement

- Non-animal methods – cell/tissue cultures, human volunteers and computer modeling

Refinement

- Alleviate or minimize potential pain, suffering or distress
- Enhance animal welfare for animals that cannot be replaced
- Improved methods with reduced variability

Reduction

- Obtain comparable amounts of information from fewer animals
- More information from same number of animals

Basic Requirements for Successful Development of Alternative Methods

1. Solid understanding of basic biology
mechanistic understanding of effect (injury)
2. In vitro platforms available
 - ∅ can be modified to make them amenable for toxicity testing
3. Scientific/regulatory community needs to be convinced
 - ∅ which is skeptical by nature and training (and rightfully so!)
 - ∅ alternative methods fulfill their intended purpose and have been rigorously validated

Basic Requirements – Mutagenicity Testing as Successful Example

- *In vitro* mutagenicity screening methods have been used for many years
è a good illustration of these three points
1. Basic biology
 - DNA is molecular basis for heredity
 - Mutations are manifestations of damage to DNA
 - Several types of mutations (e.g., point mutations, insertions, deletions) require development of different *in vitro* models
 2. *In vitro* platforms
 - molecular biology of prokaryotes, and later, eukaryotic cells
→ adapted through extensive research
 3. Years of assay standardization, replication of results in multiple laboratories, and comparisons with *in vivo* results
→ convinced scientific/worldwide regulatory community

International Efforts – Organizations

During last 2-3 decades, substantial efforts towards development and international acceptance of alternative methods to animal safety studies

- ecopa - European Consensus Platform on 3R Alternatives to Animal Experimentation
- EPAA - European Partnership for Alternative Approaches to Animal Testing
- ECVAM - European Centre for Validation of Alternative Methods
- Estiv - European Society of Toxicology In Vitro
- OECD - Organisation for Economic Co-operation and Development
- ICCVAM - Interagency Coordinating Committee on the Validation of Alternative Methods (US)
- NICEATM - NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (US)
- ICH - International Conference of Harmonization
- ILSI - International Life Sciences Institute
- IVTIP - In Vitro Testing Industrial Platform (1993)
- NC3R – UK-National Centre for Replacement, Refinement and Reduction of Animals in Research
- PSTC - Predictive Safety Testing Consortium
- ZEBET - Zentralstelle zur Erfassung und Bewertung von Ersatz- und Ergänzungsmethoden zum Tierversuch (1989)

ecopa - European Consensus Platform on 3R-Alternatives

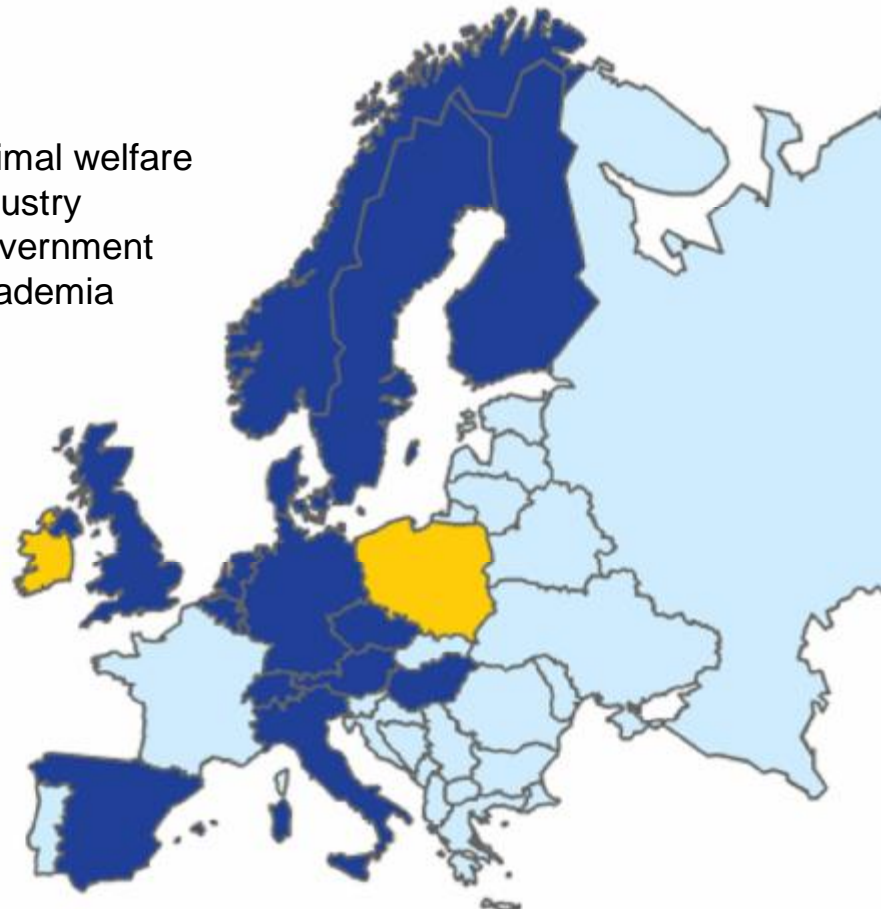


click on a country for further informations.

16 NCPs (National Consensus Platforms)
14 full member countries,
2 associate member countries

4 Parties

1. Animal welfare
2. Industry
3. Government
4. Academia



■ Full member state
■ Associated platform

AIMS

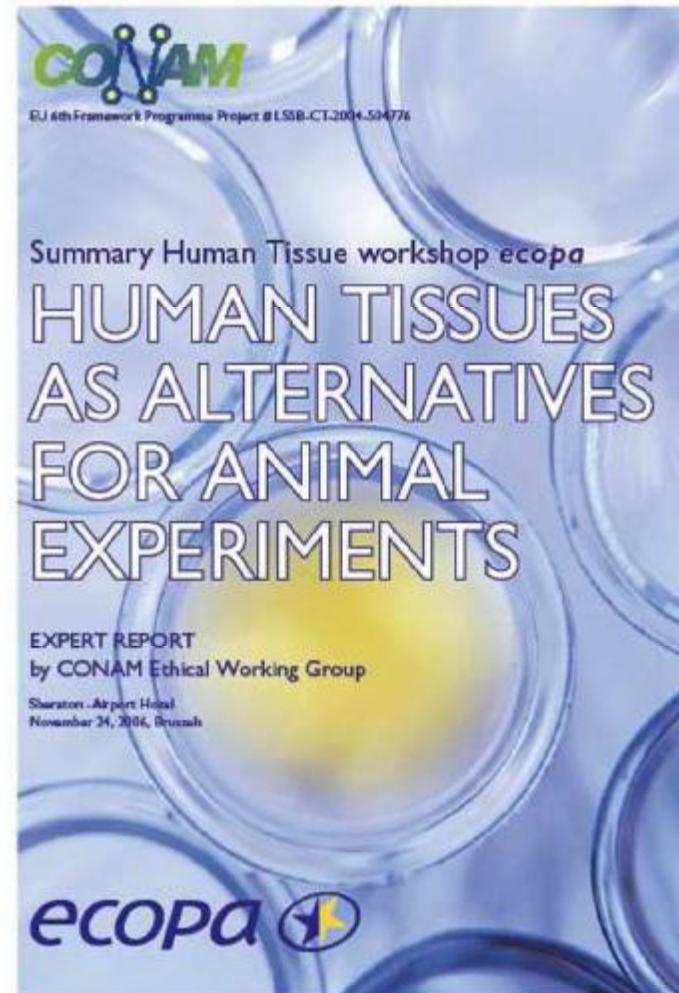
Exchange of Scientific Information, Expertise and Experience

Further Development and Implementation of 3R-Methods

Improve Public, Governmental and Scientific Awareness

- Refine
- Reduce
- Replace

CONAM (FP6) - ecopa



CONAM

Consensus
networking on
alternative
methods within
Europe

Proposed by
Ecopa

è Consensus
paper to advice
EU decision
making process

PJK
Peter-J Kramer

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OSIRIS* - Goals



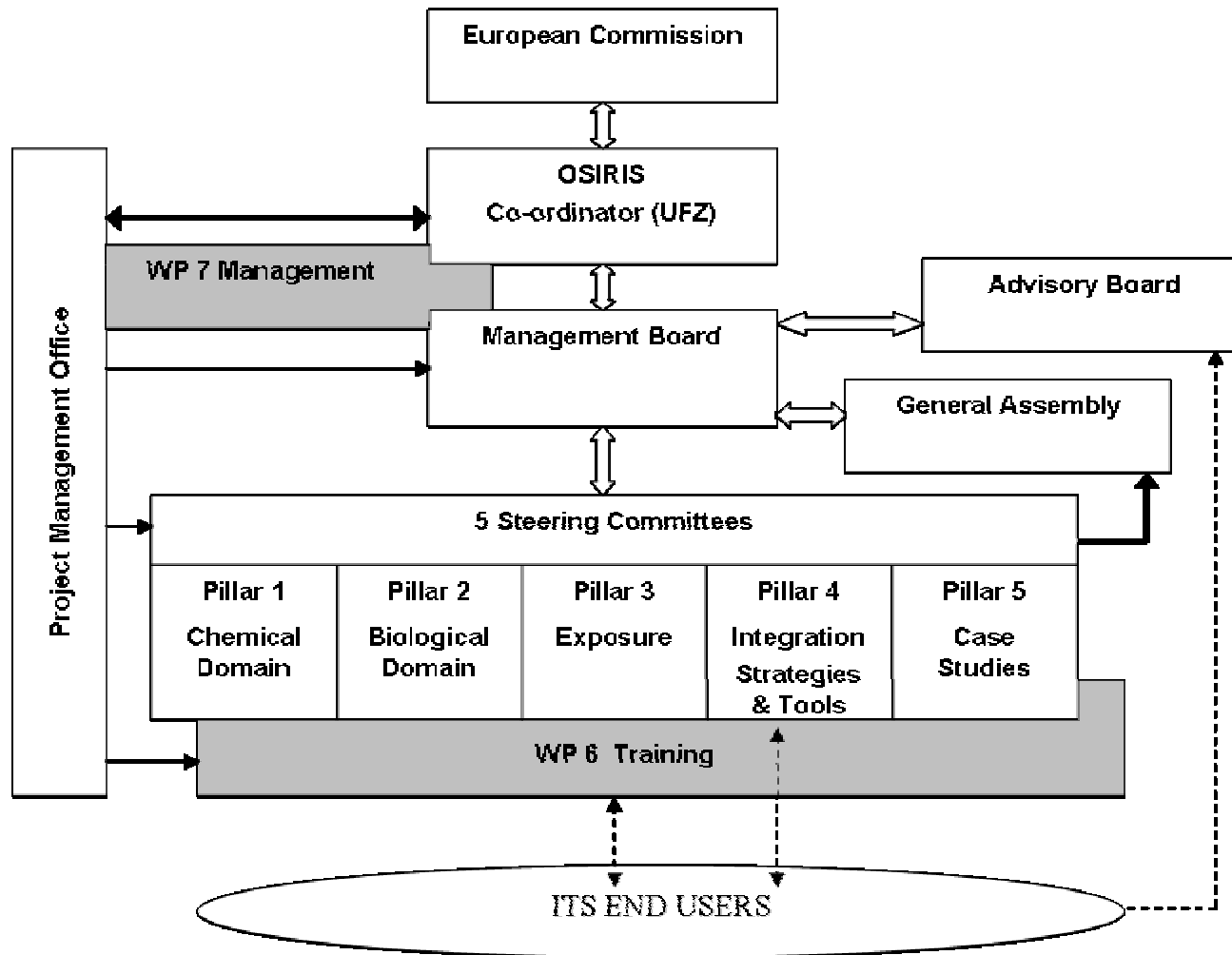
Klaus Olejniczak Symposium, Bonn , 13th December 2010

è Integrated Testing Strategies (ITS)

- Molecular structure è predicting fate and effect (in silico techniques, QSAR) (CEASAR Project)
- Mechanism-based screens (in vitro testing, optimization of in vivo protocols and mechanism-targeted genomics)
- Use patterns and conditions of use (exposure scenario)
- Weight-of-evidence approach based on computerized 'decision theory framework', incl. thresholds of toxicological concern (TTC)

* Optimized Strategies for Risk Assessment of Industrial Chemicals through Integration of Non-Test and Test Information (2007 – today)

OSIRIS-Structure



<http://www.osiris.ufz.de/>
<http://www.osiris-reach.eu/>

International Efforts – EU Projects



FP6 CONAM

Consensus Networking on Alternative Methods within Europe project

- **REPROTECT** (Development of new in vitro tests to replace animal experimentation in reproductive toxicology) (2004) (32 groups)
- **ACuteTox** (CytoTox-, Caco-2-, CFU-GM-Assays) (35 groups)
- **PREDICTOMICS** (Short-term in vitro assays for long-term toxicity) (14 groups)
- **SENS-IT-IV** (Novel Testing Strategies for *in vitro* Assessment of Allergens)
- **BioSim** (application of in silico modeling and simulation techniques)(2004) (40 groups)
- **carcinoGENOMICS** (in vitro methods for assessing carcinogenic potential of cpds)(20 groups)
- **LIINTOP** (Liver/Intestine Optimisation, PK/PD)(15 groups)
- **FORINVITOX** (effective technology transfer of alternative methods)
- **FP6/FP7 OSIRIS**
Optimized Strategies for Risk Assessment of Industrial Chemicals through Integration of Non-Test and Test Information (2007 – today) – ITS Development, in silico systems



SIXTH FRAMEWORK
PROGRAMME

2002 - 2006



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International Efforts – EU Projects



FP7 CORDIS 'Health'

(Community Research and Development Information Service)

- **START-UP**

Scientific and Technological issues in 3Rs Alternatives Research in The process of drug development and Union Politics)

- **ESNATS** (Stem cell based alternative toxicology testing)(reprotox, neurotox, metabolism and TK)(2008) (29 groups)

- **PREDICT IV** (Profiling toxicity of new drugs: a non animal-based approach integrating toxicodynamics and biokinetics (targets: liver, kidney and CNS) (2008) (20 groups)

è Objective: improved predictivity of non-clinical safety testing well in advance of

è animal safety testing (pre-phase I development) and

è clinical trials (phase I, II, III)



2007 - 2013

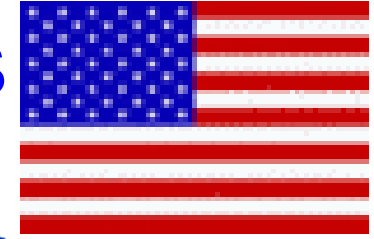
ESNATS

Embryonic Stem cell-based Novel Alternative Testing Strategies

- Goals (focus on drug development)
 - ∅ develop novel "all-in-one" toxicity test platform based on embryonic stem cells (ESCs)
 - ∅ in particular human ESC (hESCs)
 - ∅ Accelerate drug development
 - ∅ Overcome disadvantages of present approaches
 - ∅ Reduce related R&D costs
 - ∅ Alternative to animal tests (spirit of "Three R Principle")
- 4 Sub-Projects, Research Areas
 - Reproductive Toxicity, Neurotoxicity
 - Toxicogenomics/Toxicoproteomics, Metabolism/Kinetics/Modeling

International Efforts – Organisations

US Big Think



US National Research Council (NRC)

“Toxicity testing in the 21st century: a Vision and a Strategy”

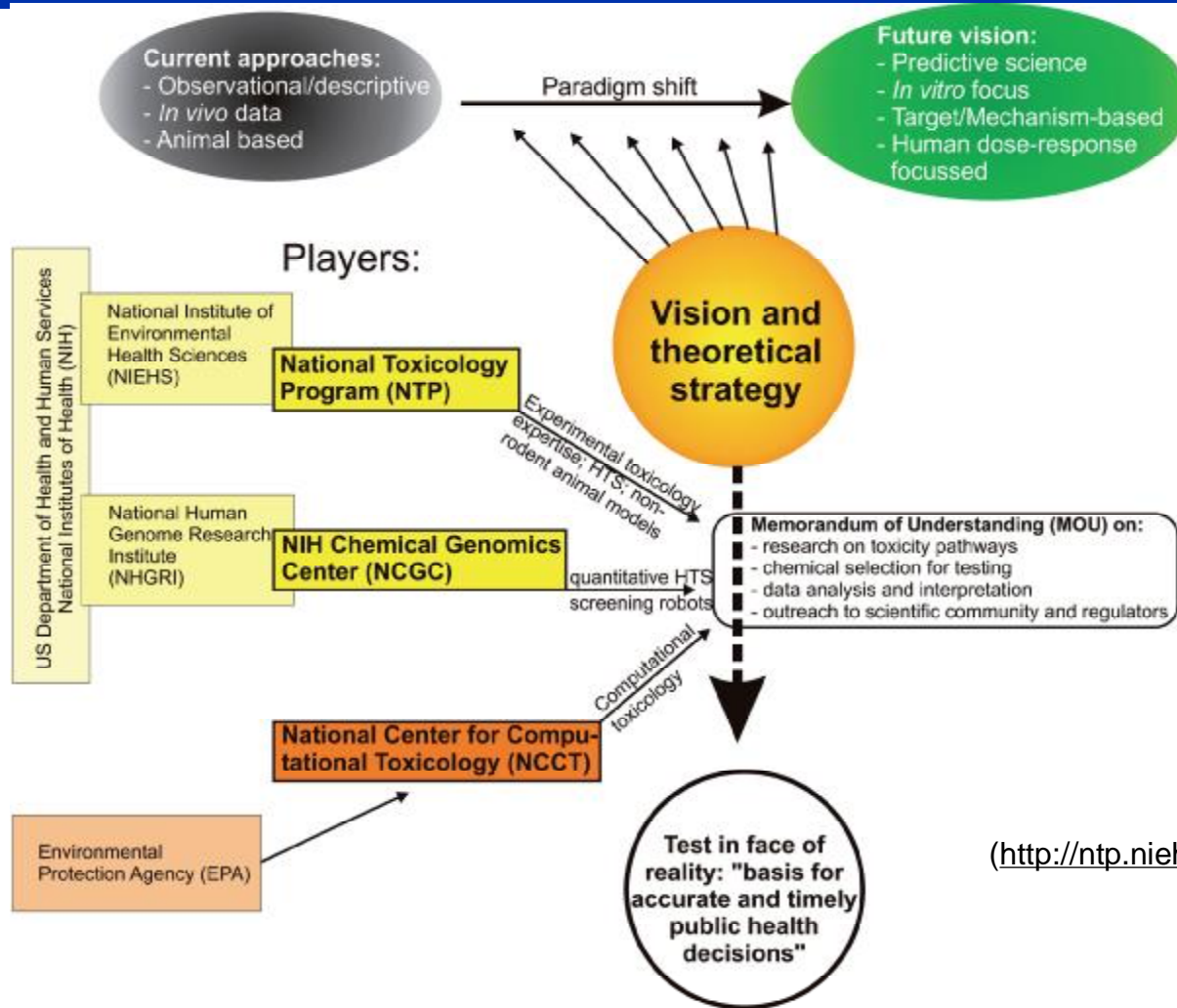
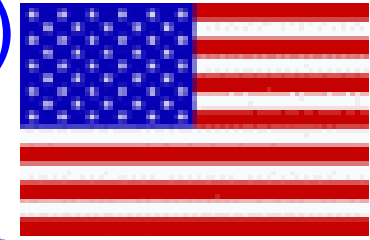
∅ Alliance formed by

- National Toxicology Program (NTP)
- Chemical Genomics Centre (NCGC)
of National Institutes of Health (NIH)
- Computational Toxicology Centre (NCCT)
of Environmental Protection Agency (EPA)

∅ Radically new approaches / new paradigm

è Future: strength of in vitro and in silico approaches based on human material

US - National Toxicology Program (NTP) for the 21st century



(<http://ntp.niehs.nih.gov/files/NTPrdmp.pdf>)



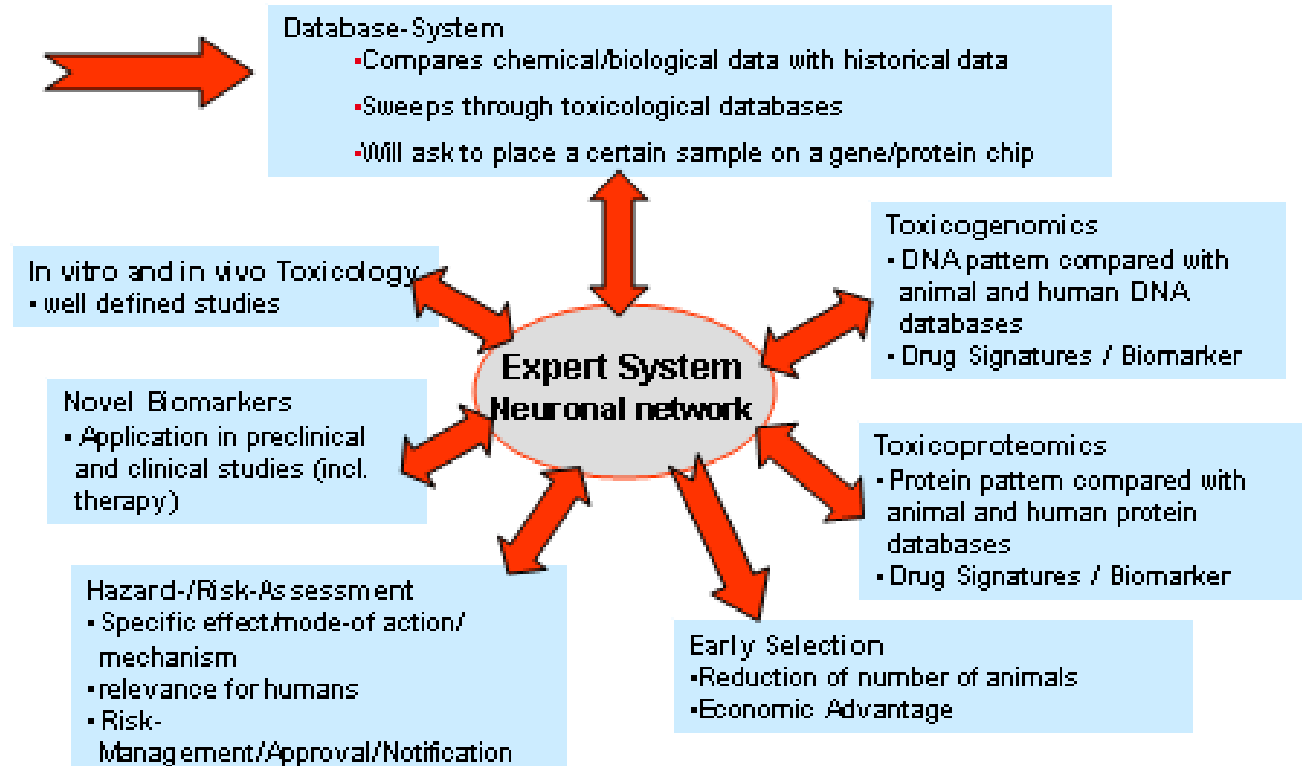
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PJK Vision in 2002

Future Industrial Toxicology



„Historic“
PJK Slide
2002



Alternative Methods - General Situation

Lilienblum et al, 2008*

- Considerable progress in establishment of alternative methods has been made in some fields, in particular with respect to methods predicting local toxic effects and genotoxicity
- Systemic single and repeated dose toxicity, toxicokinetics, sensitisation, reproductive toxicity and carcinogenicity, goal not yet reached
 - Development and validation of
 - *in silico* methods,
 - testing batteries (*in vitro* and *in silico*) and
 - tiered testing systems
 - will have to overcome many scientific and regulatory obstacles
 - Extremely difficult to predict outcome and time needed

* Alternative methods to safety studies in experimental animals: role in the risk assessment of chemicals under the new European Chemicals Legislation (REACH)
Lilienblum W, Dekant W, Foth H, Gebel T, Hengstler JG, Kahl R, Kramer PJ, Schweinfurth H, Wollin KM.
Arch Toxicol. 2008 Apr;82(4):211-36

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Why still „extremely difficult“?

Main reasons are

- Complexity of ‘Basic Biology’
 - è limited knowledge of
 - Mechanisms of biological processes
 - Interactions between systems
 - ∅ Biology is more complex than flying to the moon
- Long time frame until validation and regulatory acceptance of an alternative method

The special case of cosmetics



- n EU: challenging timelines for phasing out of many standard tests using laboratory animals were established in seventh Amending Directive 2003/15/EC to Cosmetics Directive 76/768/EEC
- § Since 11 March 2009 cosmetic ingredients are not anymore allowed to be tested on animals within EU (“testing ban”)
- § Exceptions: repeated dose toxicity, developmental toxicity and toxicokinetics
Deadline: 11 March 2013
- § From 11 March 2013 onwards all animal testing is banned
products tested on animals, even outside Europe, are completely banned from EU market (“marketing ban”)
- ∅ Directive: “Deadlines for both the testing ban and the marketing ban will apply irrespective of the availability of alternative non-animal tests.”

http://europa.eu/legislation_summaries/consumers/product_labelling_and_packaging/l21191_en.htm

The Special Case of Cosmetics



- Ban on testing of cosmetics is, at least partly, a consequence of extensive lobbying by animal welfare organizations often supported by scientists
- Most people only think of “make-up products” when cosmetics are mentioned
- However, cosmetics consist of a broad group of products extensively used and having in many cases a preventive action on disease (e.g. UV-filters to prevent skin cancer)
- è Safety control is important also for cosmetics and science should keep being involved with development of alternatives
- è Lobbying for using a methodology which is not yet ready is dangerous and bad practice
- è Pharmaceuticals must be spared of this type of lobbying

Regulatory Action

- In continuation of Cosmetic policy, also new European Chemicals Legislation (REACH) favours alternative methods to conventional *in vivo* testing
- Even alternative methods in status of prevalidation or validation, but without scientific or regulatory acceptance may be used under certain conditions!
 - Act of Desperation?
- Also ICH tries to establish some ‘alternative’ policy

ICH M3 (R2)



M3 Quote:

- “Although not discussed in this guidance, consideration should be given to use of new *in vitro* alternative methods for safety evaluation”
 - “These methods, if validated and accepted by all ICH regulatory authorities, can be used to replace current standard methods”
 - TK/PK data: “In vitro metabolic and plasma protein binding data for animals and humans ... *in vitro* biochemical information relevant to potential drug interactions should be available before exposing large numbers of human subjects or treating for long duration”
- è ICH is still very careful, but slowly opens the door

<http://www.ich.org/LOB/media/MEDIA5544.pdf>

Example - Eye Safety Testing

Reason for testing

- Protect workers and consumers from temporary or permanent eye injuries such as blindness
- e.g. U.S.A.: ~ 2 million eye injuries / year
 - more than 40,000 result in permanent visual impairment
 - Over 15% of such injuries (6.000) are related to household cleaning chemicals and other chemical products

Ø Regulation:

Testing required to determine if chemicals and products may cause such damage

Eye Safety Testing – Draize Test

- Eye irritation and corrosion
 - Supposedly relatively simple target for replacement
 - However, extensive list of alternative *in vitro* models
- Why extensive list?
 - Because even supposedly simple targets for replacement have proved difficult to be modeled in vitro
 - Slow progress and difficult validation despite major efforts, e.g. by ECVAM (European Centre for the Validation of Alternative Methods), industry and academia

Draize Test Alternatives- 4 Categories

1. Target organ/tissue assays

- Bovine corneal opacity and permeability (BCOP) test
- Isolated rabbit eye (IRE) test
- Chicken enucleated eye test (CEET)

2. Organotypic models

- Hen's egg test–chorioallantoic membrane (HET-CAM) assay
- Chorioallantoic membrane vascular assay (CAMVA), tissue equivalent assay

3. Cytotoxicity assays

- Neutral red assays (neutral red uptake, fibroblasts)
- Red blood cell lysis assay (cell membrane lysis)
- Fluorescein leakage test (FLT)(epithelial cell monolayers)

4. Chemical reaction assays

- Irritation Assay System (IAS) (corneal proteins)

Validation of Draize Alternatives

- **Six major validation or evaluation studies** conducted between 1991 and 1997 in different locations
 1. European Commission/British Home Office Study (Balls et al. 1995)
 2. European Cosmetic, Toiletry and Perfumery Association (COLIPA) study (Brantom et al. 1997)
 3. Bundesgesundheitsamt/German Department of Research and Technology (BGA/BMBF) study (Spielmann et al. 1993, 1996)
 4. United States, Cosmetics, Toiletries and Fragrance Association (CTFA) study (Gettings et al. 1991, 1994, 1996)
 5. US-Interagency Regulatory Alternatives Group (IRAG) study (Bradlaw et al. 1997)
 6. Japanese Ministry of Health and Welfare/Japanese Cosmetic Industry Association (MHW/JCIA) study (Ohno et al. 1994)
- Ø “None of the methods included in these validation/evaluation studies met all formal validation requirements of regulatory authorities for replacing the current animal test for acute eye irritation/corrosion” (OECD 2002)

US-ICCVAM - Eye Safety Testing

US Interagency Coordinating Committee on Validation of Alternative Methods (ICCVAM)

Federal Register Vol. 75, No. 180, September 17, 2010*

”Recommendations to Provide for More Humane Eye Safety Testing”

- Always use topical anesthetics, analgesics, and humane endpoints
- Whenever necessary to use animals avoid pain and distress
- No *in vitro* test and no *in vitro* test battery can yet be recommended for regulatory use

è Consequence for cosmetic’s time-frame in EU?

* <http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/FR/FR-2010-23262.pdf>

Areas of highest contribution to animal (mammalian) use and costs

- Repeat dose toxicity (drugs)
- Reproductive toxicity (REACH)
 - è No relevant alternative approaches have emerged
 - è Fish larvae test?
 - è At least no method is under validation
 - è no validation / peer-review / acceptance before 2018

Ways out?

Toxicology

- è Continuous high responsibility for human safety
- è Not possible to drop precautionary principle
- è Scientific evidence remains best way to find acceptable solutions
- In vitro assays: many are still 'black box' systems
- Pharmacokinetic information: has been terribly neglected
- è Integrated Testing Strategies (ITS)
 - è combination of existing data, chemical categories/grouping, in vitro test battery and in silico, computer based assessment)
- è Could this be a way out?

Future Prospect (1)

Unprecedented large and ambitious Initiatives have been started

- US National Research Council (NRC)
“Toxicity testing in the 21st century: a Vision and a Strategy”
- EU FP6/FP7 projects
 - OSIRIS - Optimized Strategies for Risk Assessment of Industrial Chemicals (ITS)
 - START-UP – Focus on pharmaceutical research and development
 - ESNATS - Stem cell based alternative toxicology testing (reprotox, neurotox, metabolism and TK)
 - PREDICT IV - Profiling toxicity of new drugs: a non animal-based approach integrating toxicodynamics and biokinetics (targets: liver, kidney and CNS)

è There is potential for **replacement!**

- Agreement that in vivo acute toxicity is no longer asked for drugs


è There is potential for **reduction !**
(also in repeat tox and reproduction)

Future Prospect (2)

- Large initiatives in US and EU show
 - ∅ Replacement initiatives must be accepted as ambitious long-term activities
 - ∅ Goal should be to improve predictivity, especially regarding quantitative aspects of risk assessment such as NOAELs, LOAELs and health-related or clinically relevant decisions
 - ∅ Replacement must provide at least same level of protection of human health
 - ∅ Otherwise in vitro/in silico methods will have only moderate impact on reduction of in vivo tests
- Short- and mid-term strategy will therefore include refinement or reduction of in vivo tests

Conclusion

- è Today animal experiments are still needed
- è Realistic progress is actually expected by intelligent combination of refinement, reduction and replacement methodologies and strategies
- è *In vivo* and *in vitro* research and testing should go together and not be seen as two opposites
- è Both sides must focus on best science for best toxicology
- è It should never be forgotten: reality for humans is what one strongly believes, even in toxicological science !



Klaus,
thanks a lot for
many important contributions.
For being a critical but always
forward-looking colleague.



