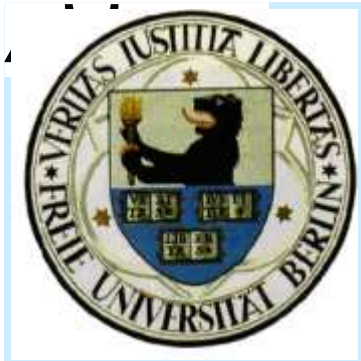


Risiken erkennen – Gesundheit schützen

JSAAE & JaCVAM Joint Workshop
"International Trends on 3Rs in Animal Experiments"
14 February 2011, Tokyo

The FP7 Project AXLR8 – Accelerating the transition to a toxicity pathway-based paradigm for chemical safety assessment through internationally coordinated research and technology development

Dr. med. Horst Spielmann
Professor for Regulatory Toxicology
Institute for Pharmacy Freie Universität Berlin
former head of ZEBET at the BfR

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

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

Breaking News

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

Topics

I. The US toxicology in the 21st Century project

II. Incentives in Europe to reduce regulatory testing in animals:
7th Amendment of the EU Cosmetics Directive & the REACH policy

III. Funding concept of the EU FP6 & FP7 programs on alternatives

IV. AXLR8 = accelerate (coordination support project)

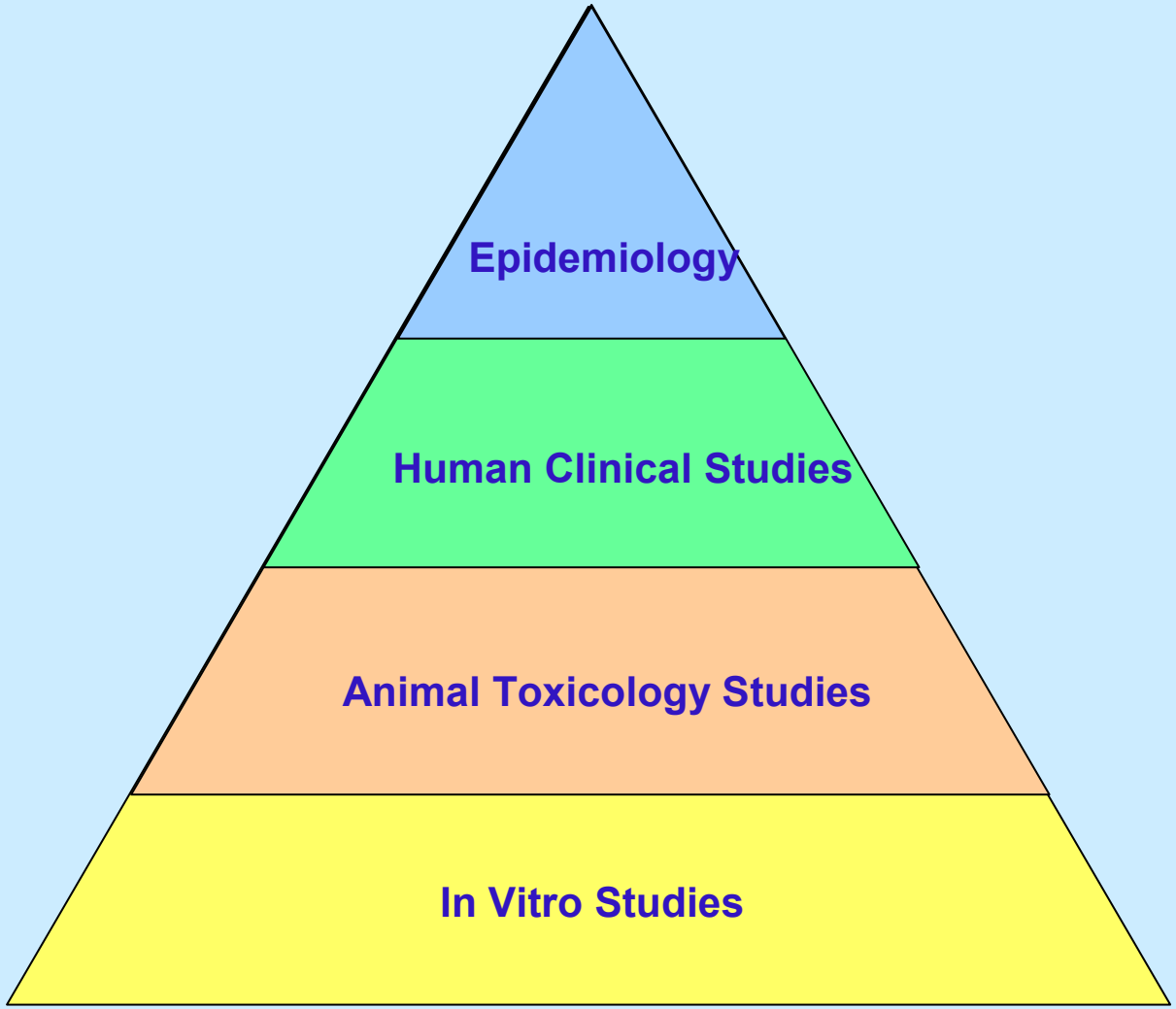
V. EU–COLIPA project on replacing systemic repeated dose toxicity

VI. Advances in reproductive toxicity testing:
a) EOGRTS: Extended One-Generation Reproductive Toxicity Study
b) EU Project ReProTect: innovative developmental toxicity testing (mEST & hEST)

VII. The future: toxicity pathway based testing (US Tox21 concept)

In Vitro Studies: What is their Role in Toxicology?

Robert Devlin US EPA 2005



AXLR8

2007

THE NATIONAL ACADEMIES

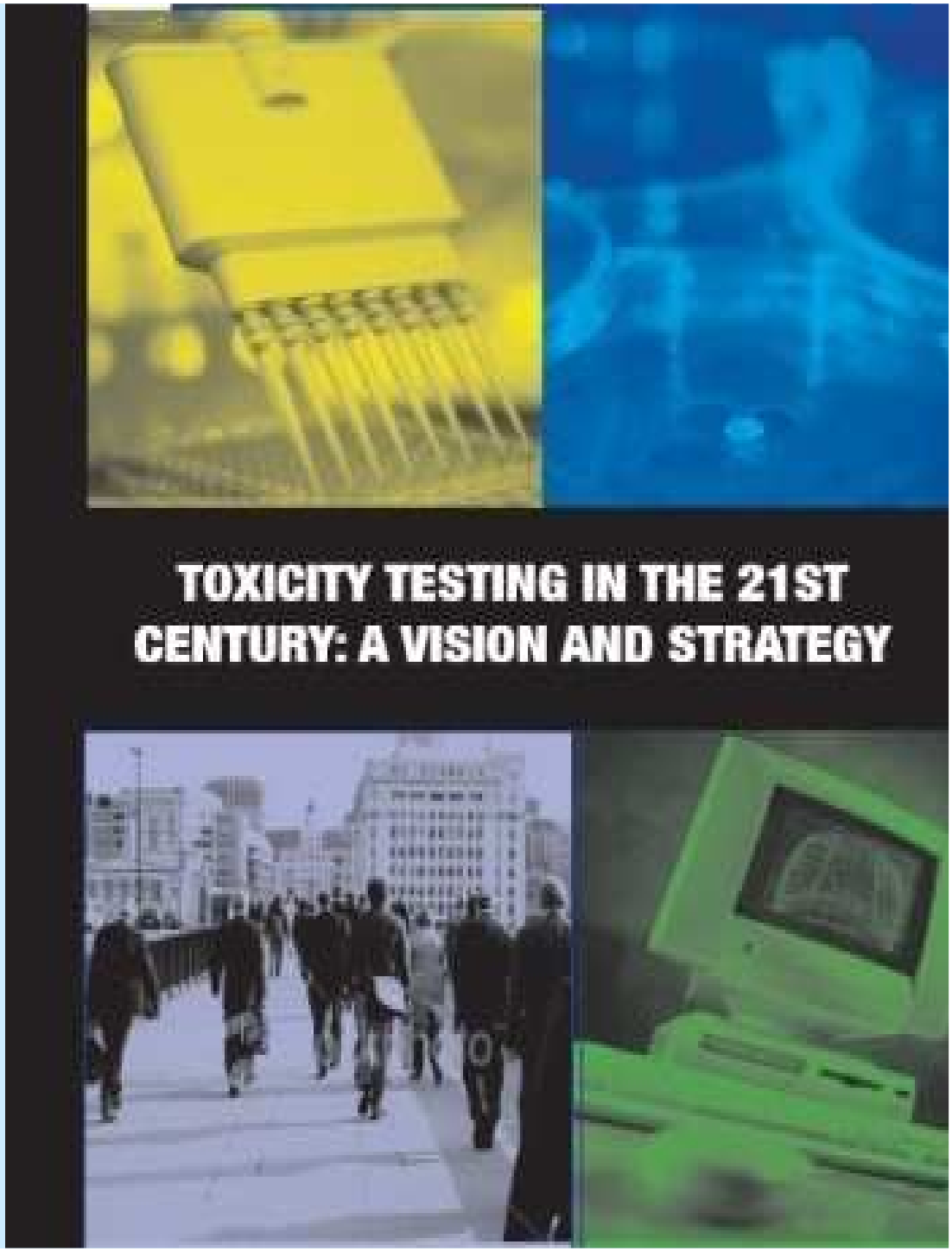
REPORT IN BRIEF

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.

US National Academy of Sciences 2007

"….. a not-so-distant future where all routine toxicity testing will be conducted in human cells or cell lines *in vitro* by evaluating perturbations of cellular responses in a suite of toxicity pathway assays….."



Andersen and Krewski (2009). Toxicity Testing in the 21st Century: Bringing the Vision to Life. Tox. Sci., 107, 324-330.

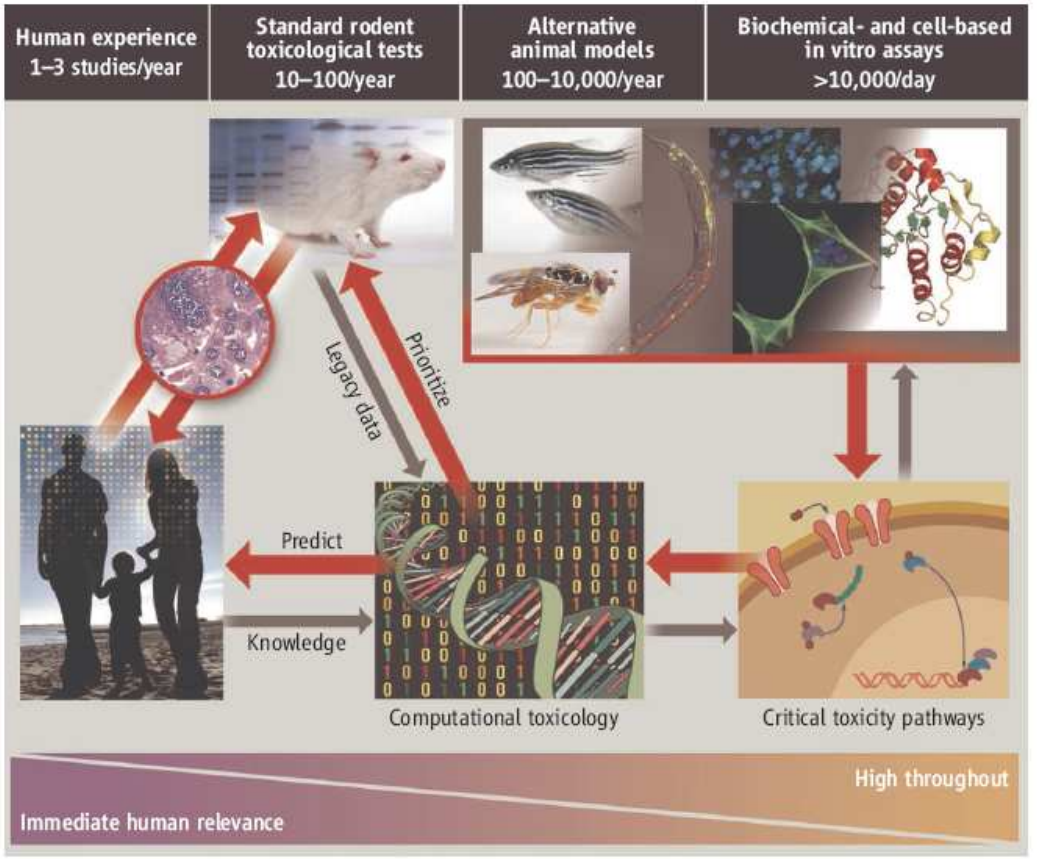
15 FEBRUARY 2008 VOL 319 SCIENCE www.sciencemag.org

TOXICOLOGY

Transforming Environmental Health Protection

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

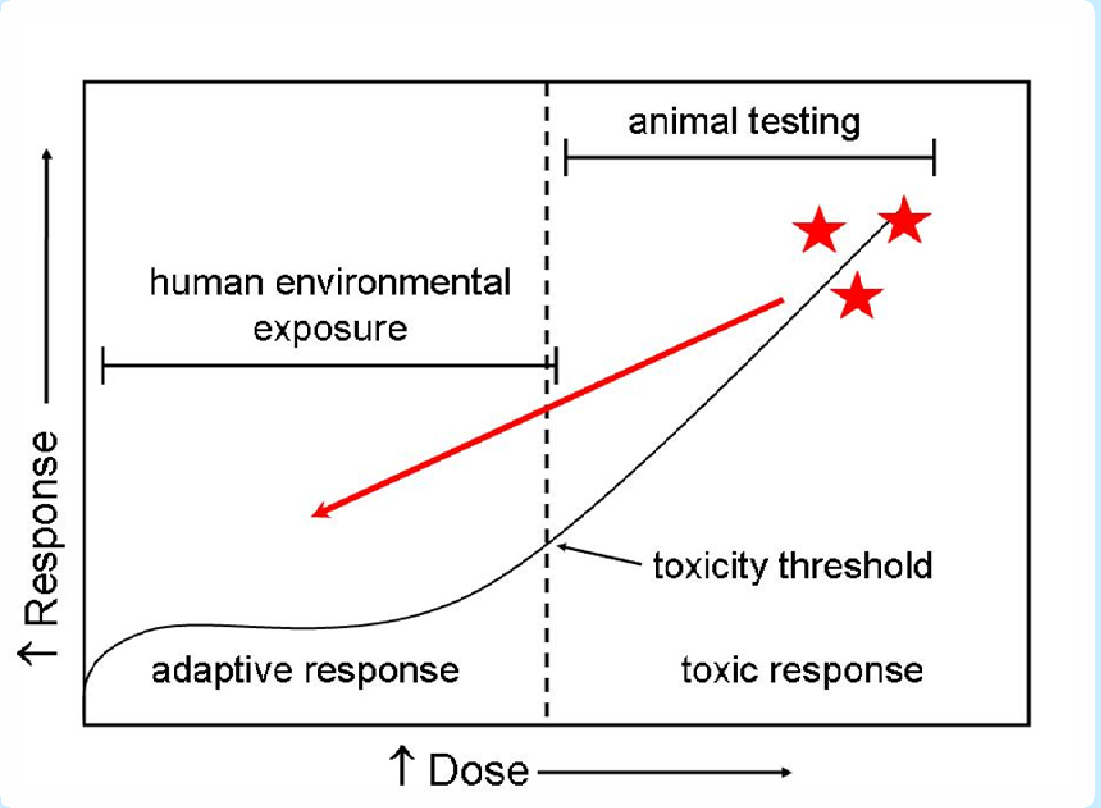
We propose a shift from primarily in vivo animal studies to in vitro assays, in vivo assays with lower organisms, and computational modeling for toxicity assessments.



Transforming toxicology. The studies we propose will test whether high-throughput and computational toxicology approaches can yield data predictive of results from animal toxicity studies, will allow prioritization of chemicals for further testing, and can assist in prediction of risk to humans.

Increasing frustration with current approaches to toxicity testing …

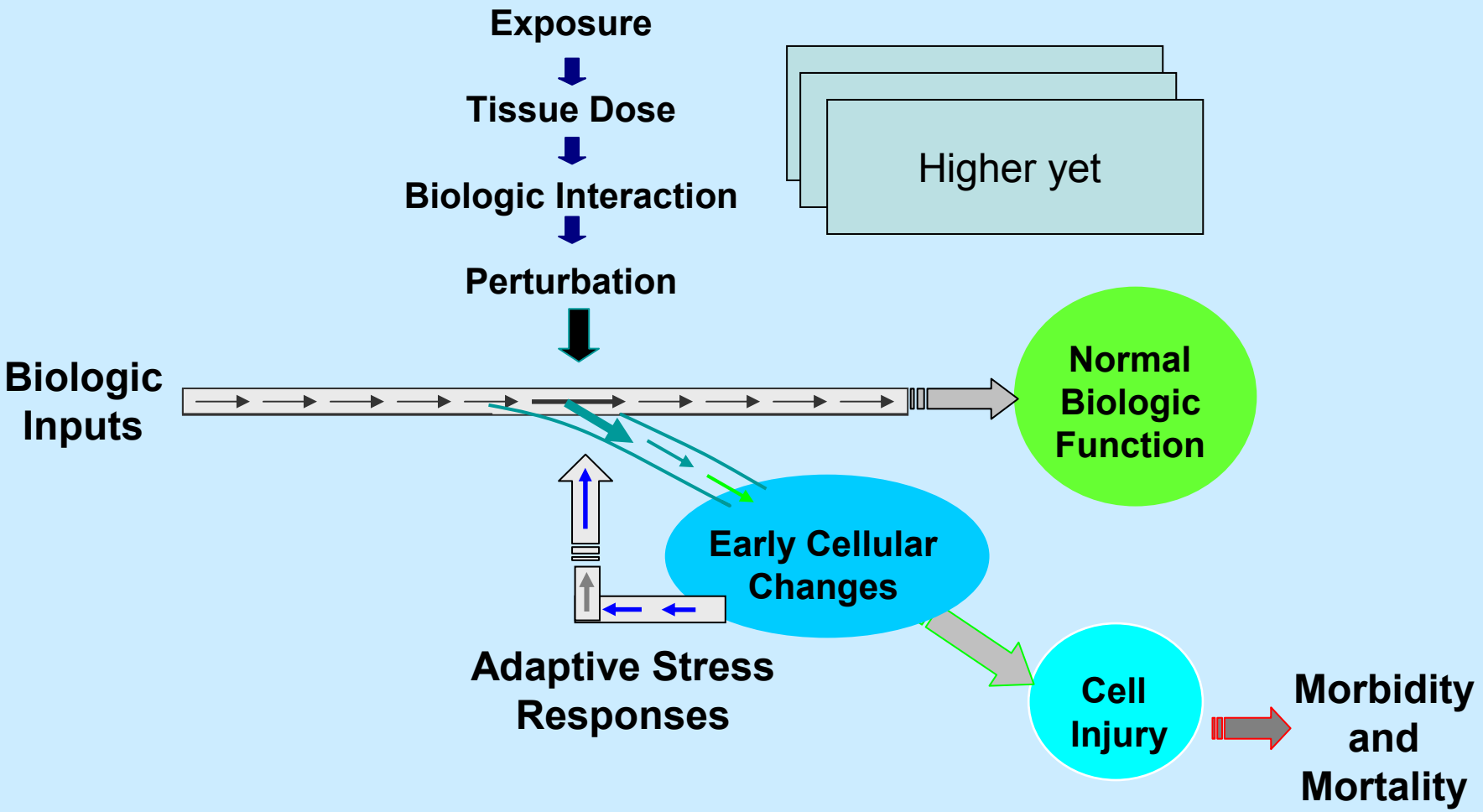
- Low throughput; expensive
- Questionable relevance to actual human risks
- Conservative extrapolation defaults, including safety factors
- Traditional approaches dating to 1930's
- Reliance on animals
- Little use of modern biology, mode of action
- Requires:
  1. Extrapolation across species
  2. Extrapolation from high-to-low dose



Options for Future Toxicity Testing Strategies

Option I In Vivo	Option II Tiered In Vivo	Option III In Vitro/In Vivo	Option IV In vitro
Animal biology	Animal biology	Primarily human biology	Primarily human biology
High doses	High doses	Broad range of doses	Broad range of doses
Low throughput	Improved throughput	High and medium throughput	High throughput
Expensive	Less expensive	Less expensive	Less expensive
Time consuming	Less time consuming	Less time consuming	Less time consuming
Relative large number of animals	Fewer animals	Substantially fewer animals	Virtually no animals
Apical endpoints	Apical endpoints	Perturbations of toxicity pathways	Perturbations of toxicity pathways
	Some *in silico* and *in vitro* screens	*In silico* screens possible	*In silico* screens

Perturbation of Toxicity Pathways



SG 1 Toxicity Pathway Identification

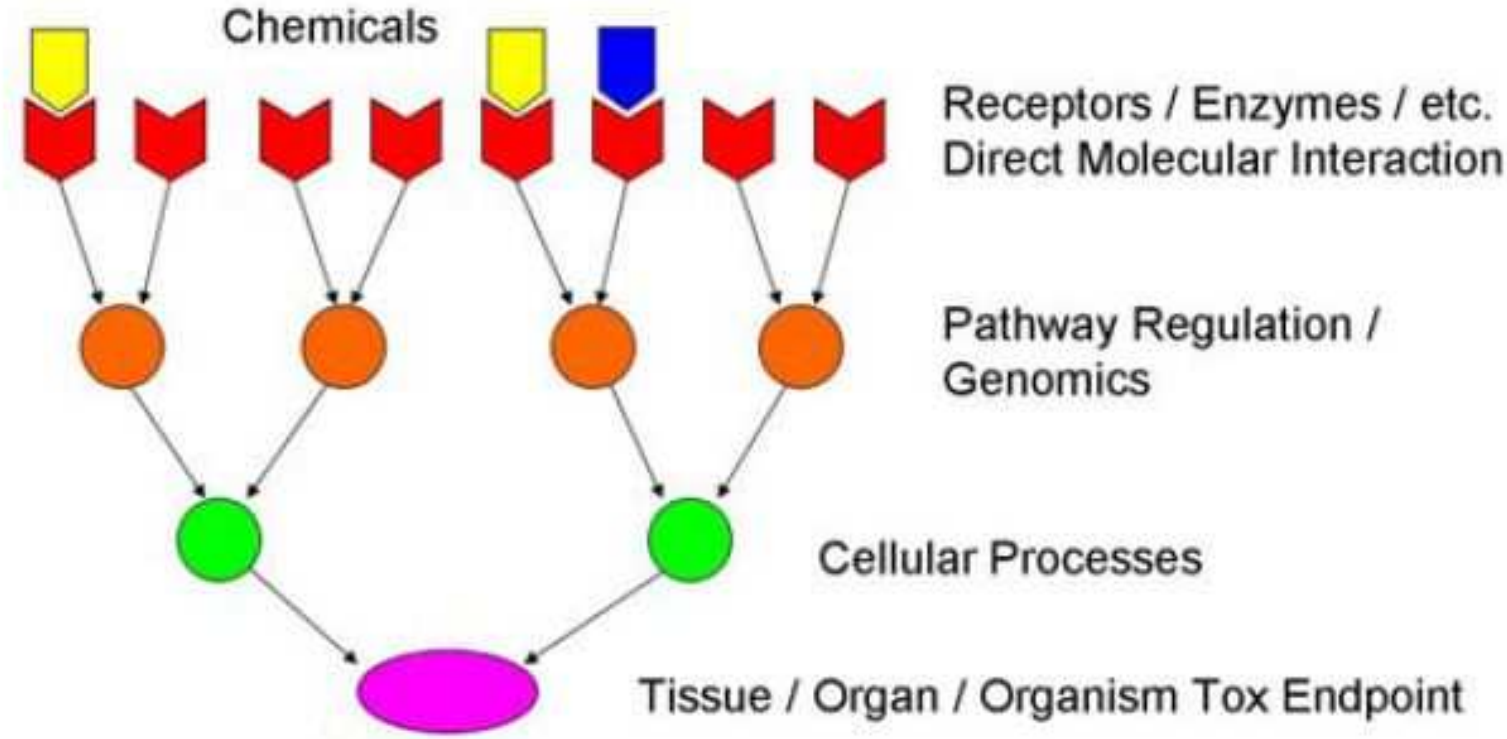
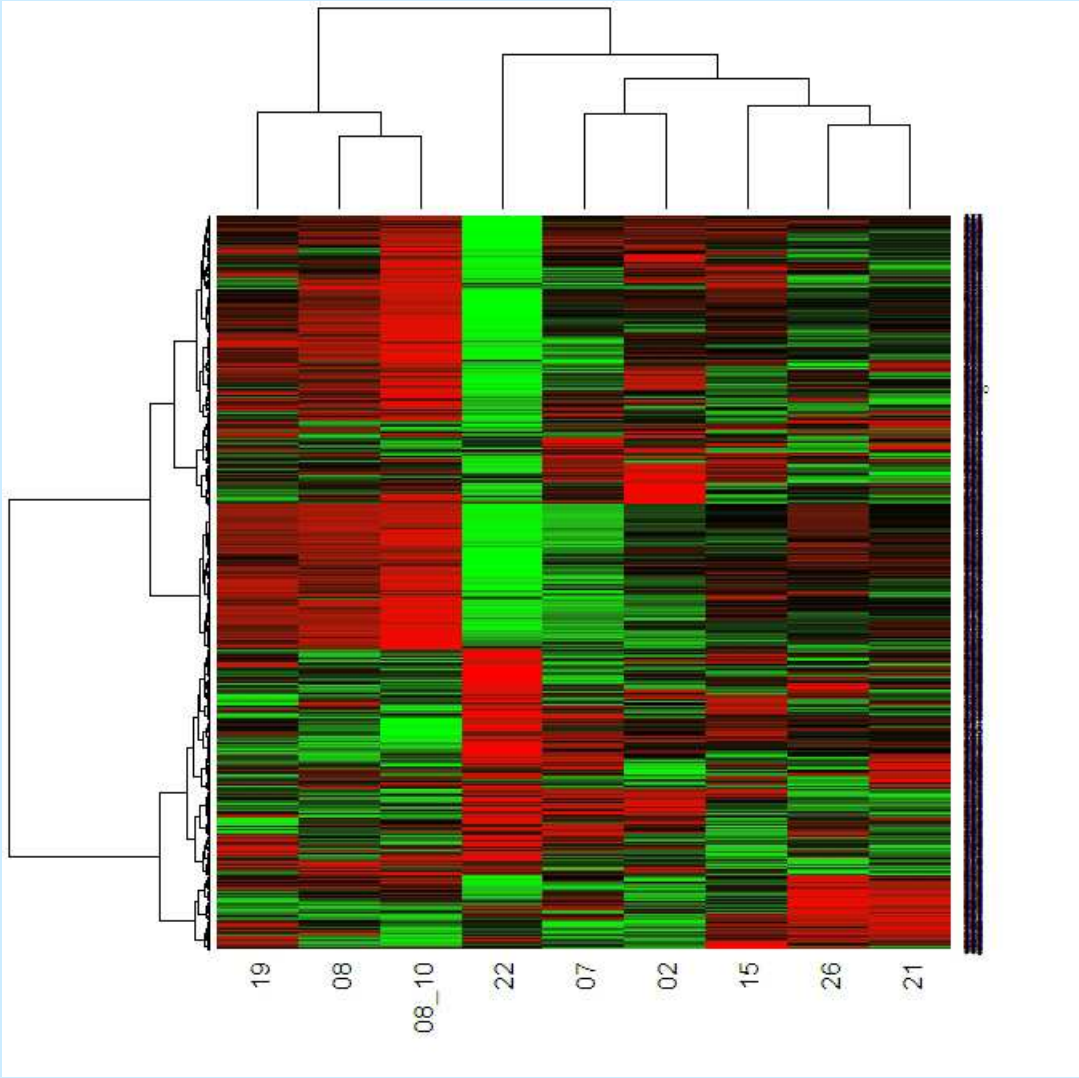


Figure 2. Toxicity Pathways Target Multiple Levels of Biological Organization.

Pathway Testing – toxicogenomics, etc.



Assess pathways, integrate tissue responses, and evaluate metabolites

SG2 Assay Development

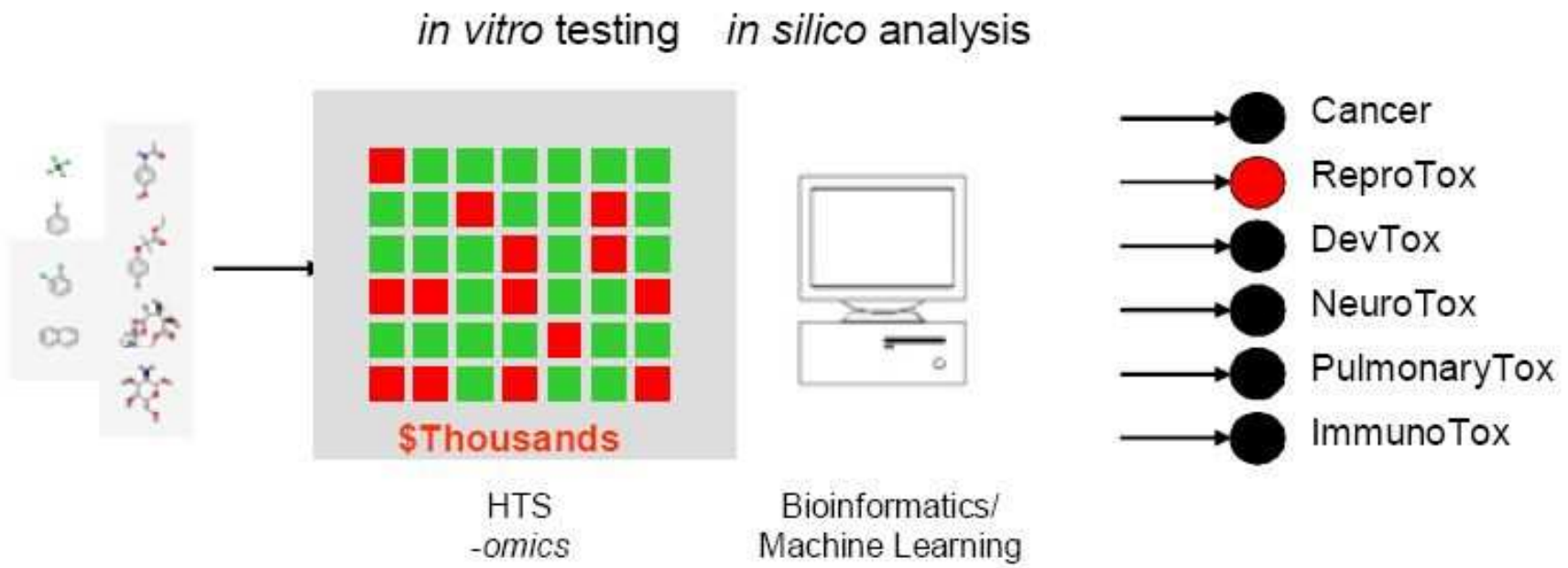
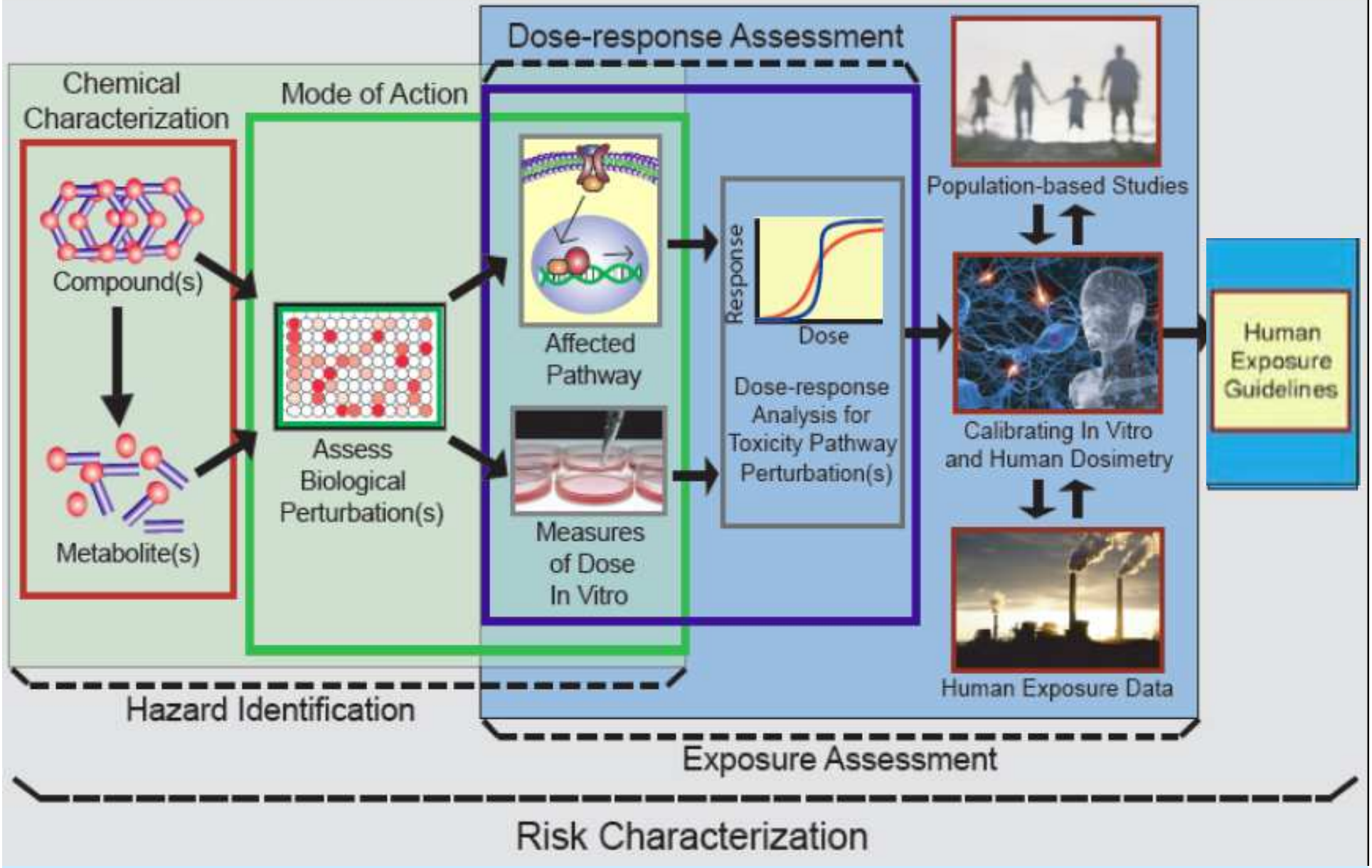


Figure 3. ToxCast™ is using a variety of HTS assays to develop bioactivity signatures that are predictive of effects in traditional toxicity testing approaches.

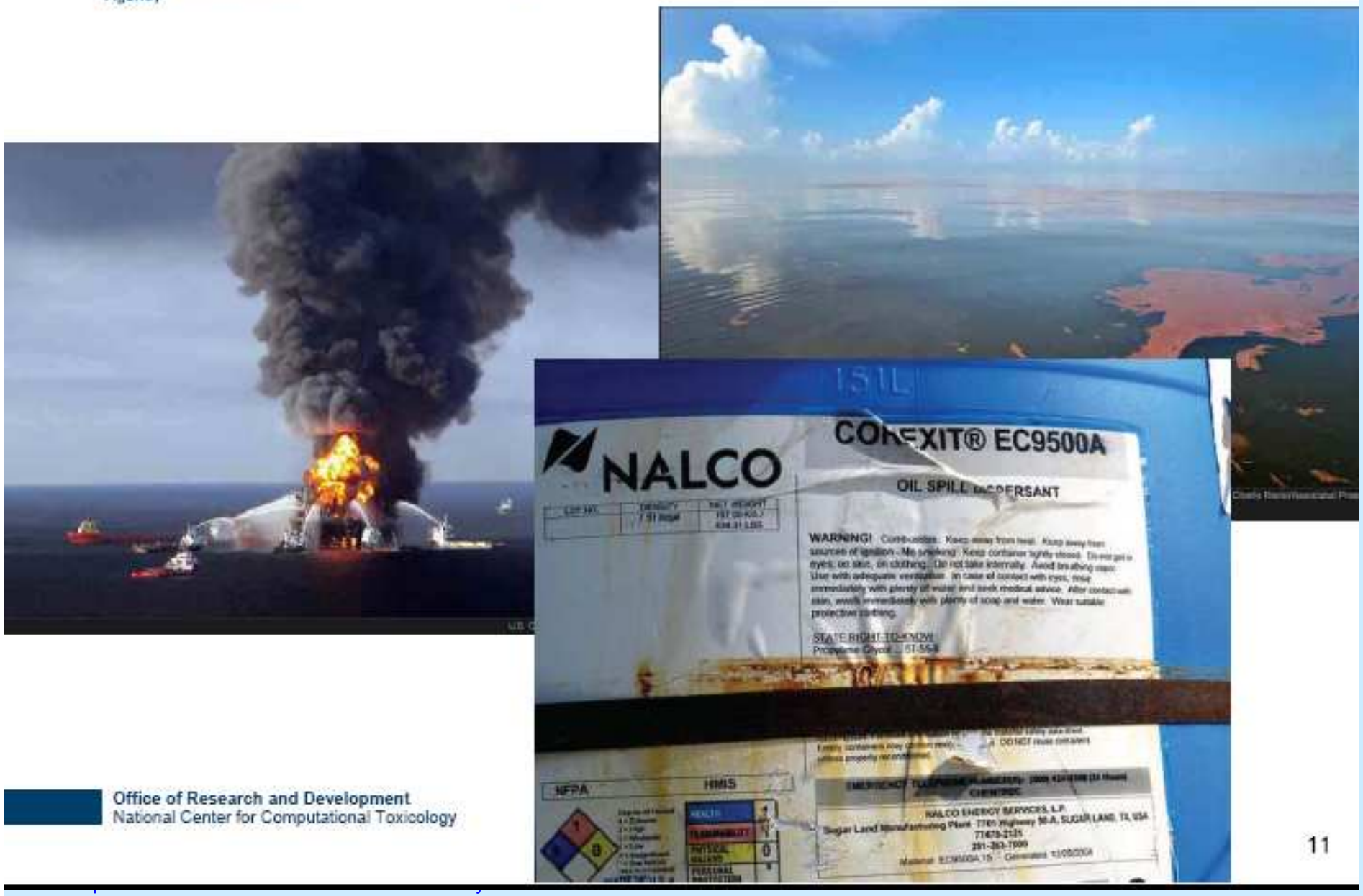
Toxicity Testing and Risk Assessment

(from Krewski et al., 2010, Annual Review of Public Health, in press)



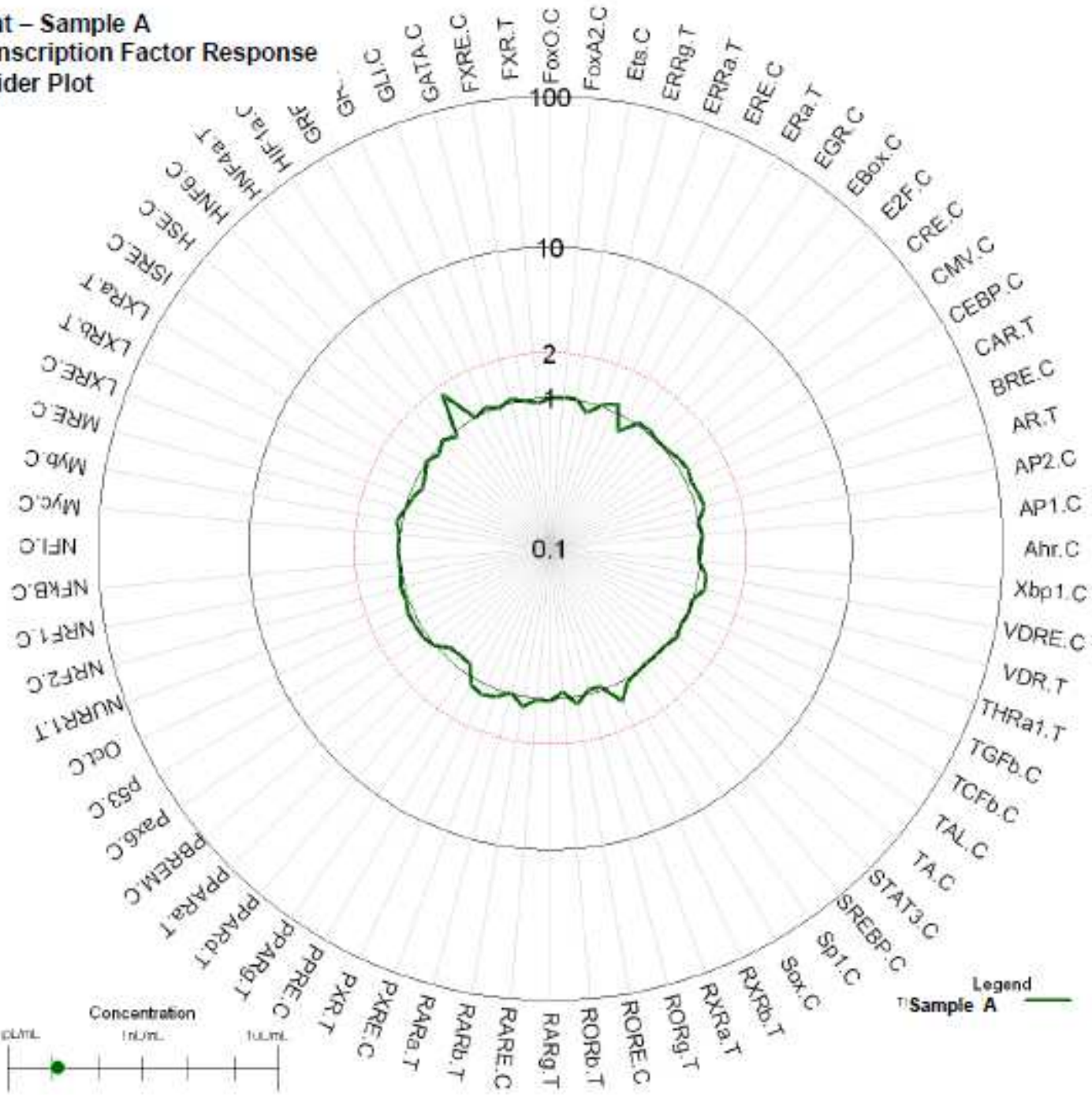
Unexpected Application: Deepwater Horizon Disaster

EPA United States Environmental Protection Agency



Office of Research and Development
National Center for Computational Toxicology

Oil Dispersant – Sample A
Attagene Transcription Factor Response
Animated Spider Plot



The Transatlantic Divide

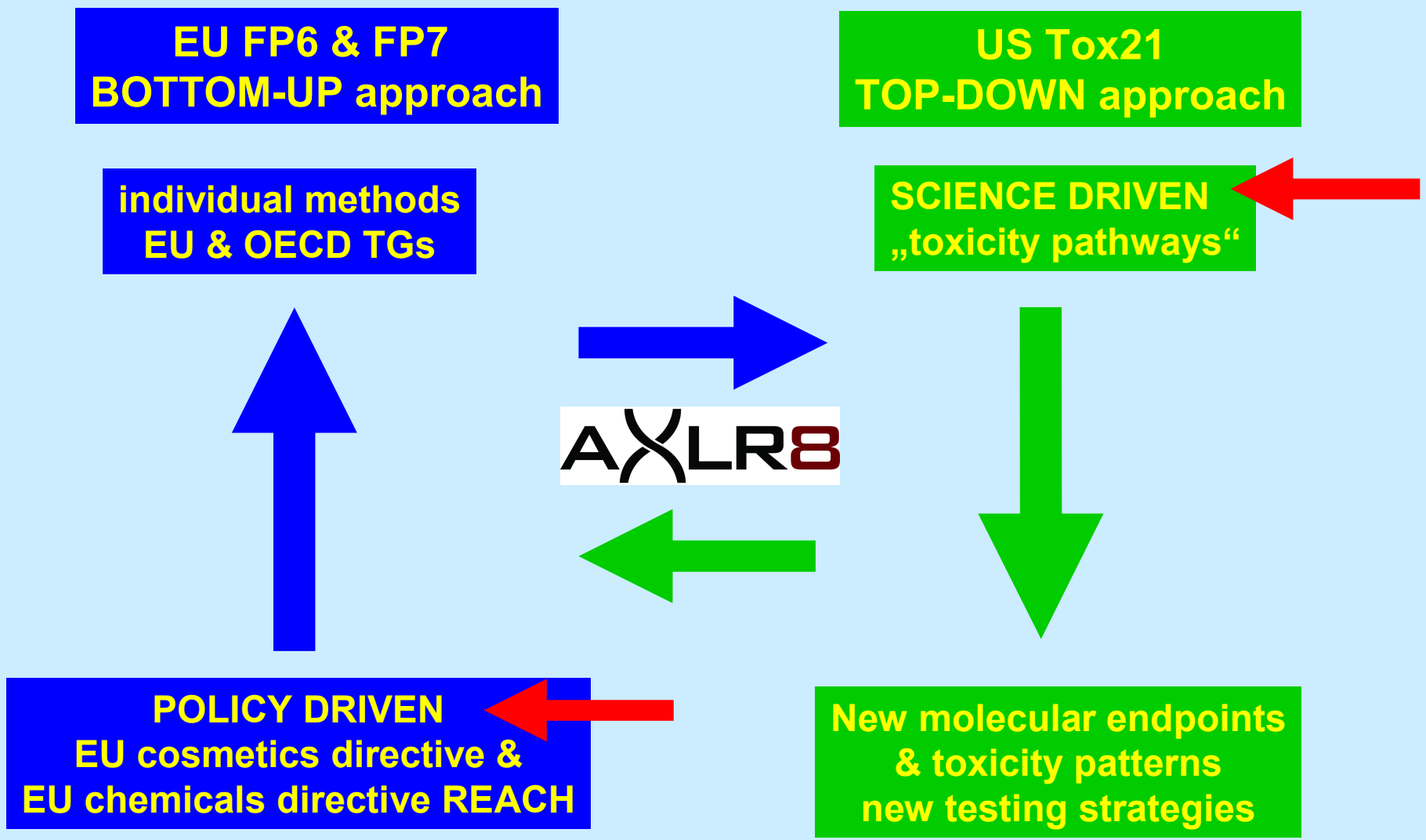
Top-down development of new toxicological tools

Tox-21c

3Rs

Bottom-up support to alternative methods and legislative pressure

EU and US approaches to advanced toxicity testing 2010



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**

7th Amendment of the EU Cosmetics Directive 76/768/EEC 2003

Ban of testing in animals

immediately ➔ since 2003

Testing of	**- finished products**
Testing for	**- phototoxic potential**
	- skin penetration
	- skin corrosion

Intensive research will be required to reach validation and regulatory acceptance of in vitro test for the following endpoints :

31. Dec. 2009	**- eye irritation**
	- skin irritation
	- skin sensitization
31. Dec. 2013	**- embryotoxicity - EST ?**

7th Amendment of the EU Cosmetics Directive 76/768/EEC 2003

Basic concept:

An alternative method has to be used for safety testing of cosmetic ingredients as soon as it is accepted by the OECD.

If in vitro methods are not available, there will is a marketing ban starting in 2010 or 2014 !

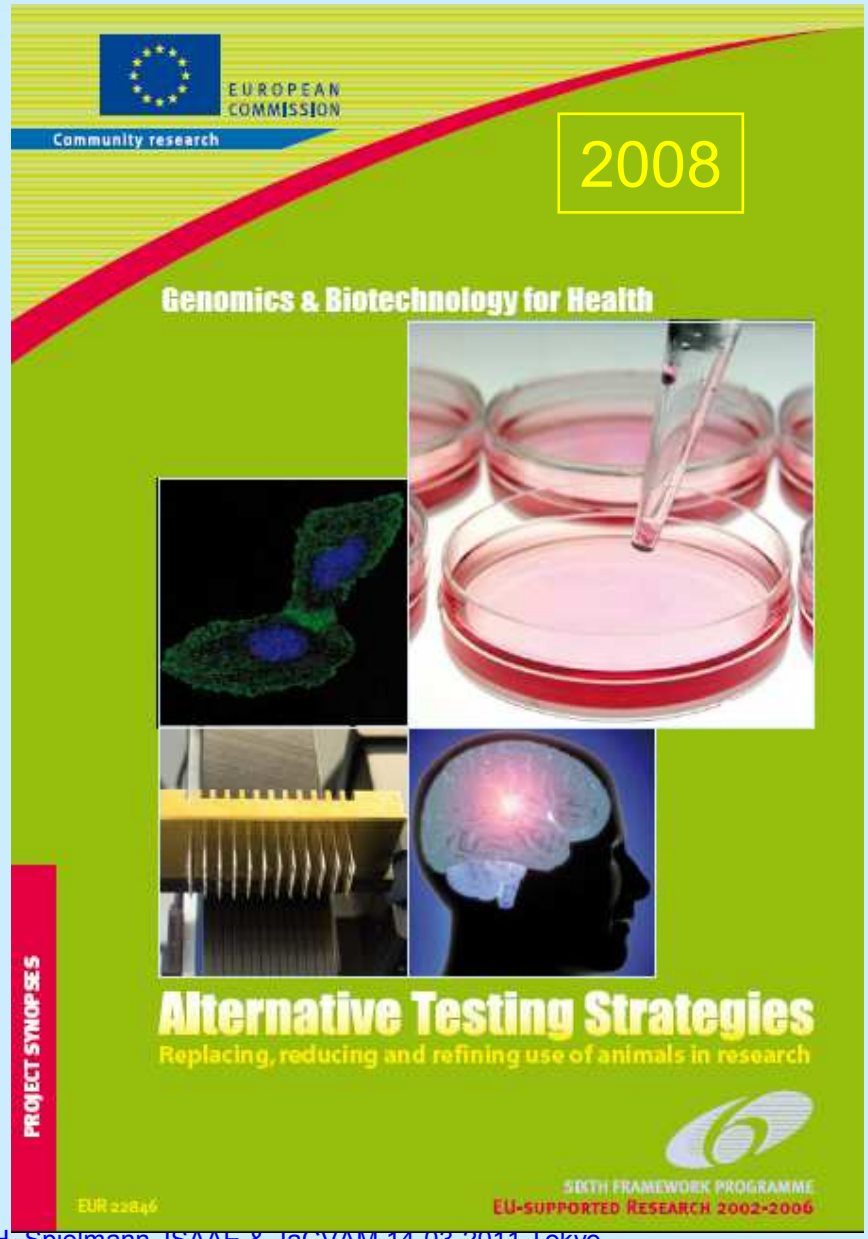
Exemptions:

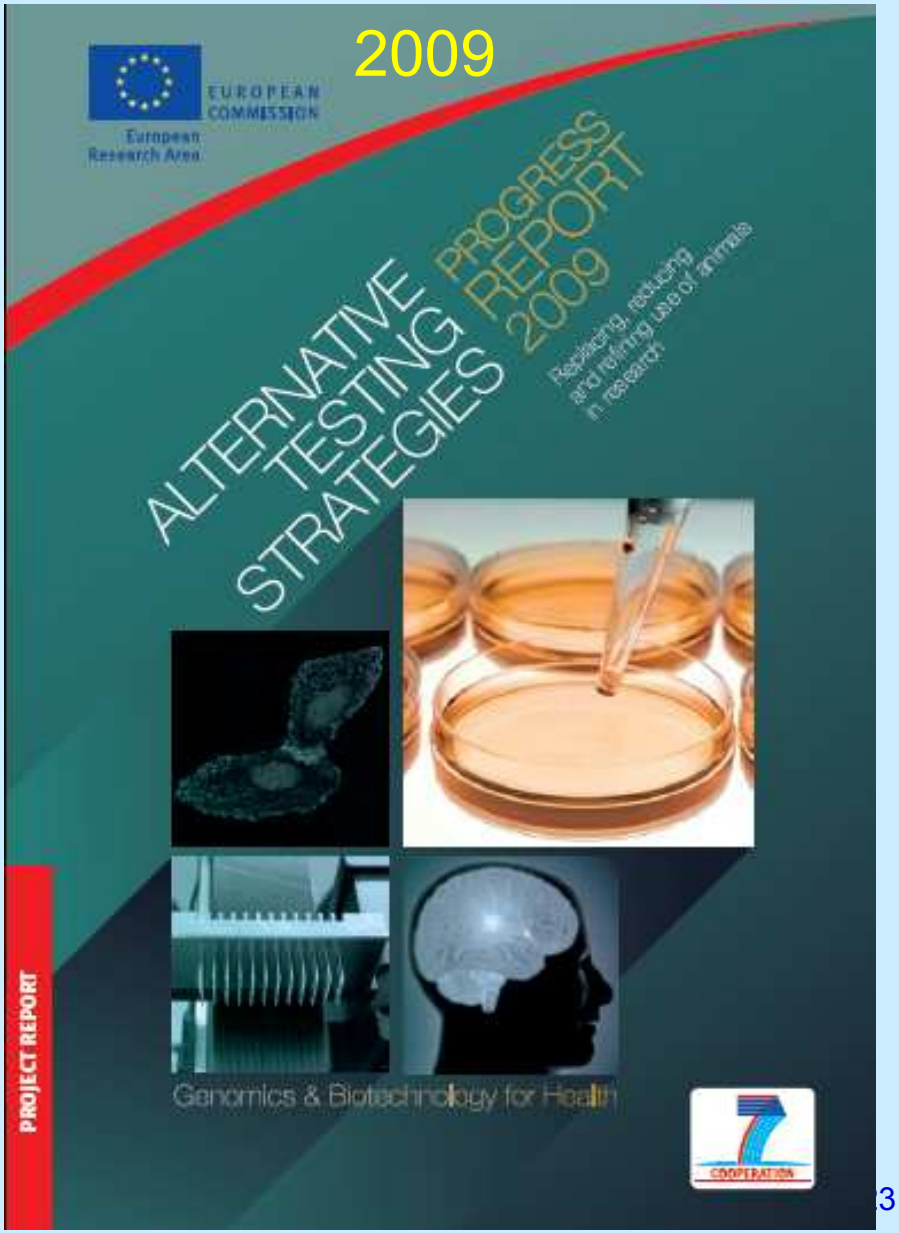
In 2013 most probably in vitro methods will not be available to test for the following endpoints:

1. **Toxicokinetics**
2. **Reproduction (fertility and embryotoxicity)**
3. **Carcinogenic potential**

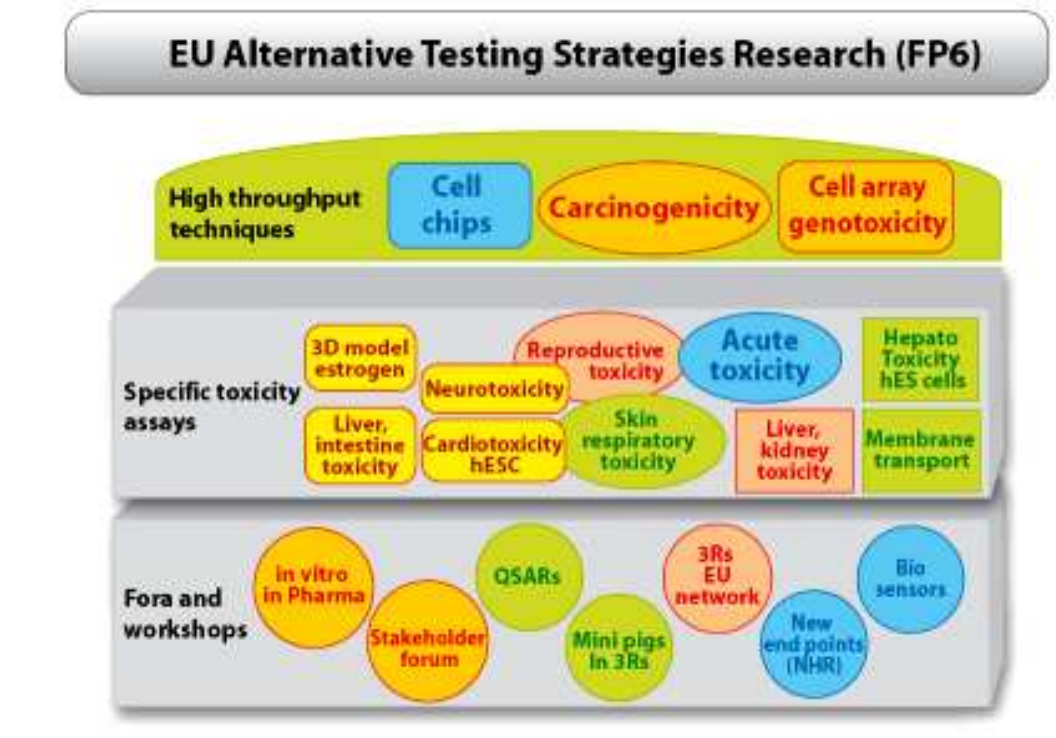
The Chemical Industry and REACH

- **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**


EUROPEAN COMMISSION
Community research
2008
Genomics & Biotechnology for Health
PROJECT SYNOPSES
Alternative Testing Strategies
Replacing, reducing and refining use of animals in research
EUR 22846
SIXTH FRAMEWORK PROGRAMME
EU-SUPPORTED RESEARCH 2002-2006


2009
EUROPEAN COMMISSION
European Research Area
ALTERNATIVE TESTING STRATEGIES
PROGRESS REPORT 2009
Replacing, reducing and refining use of animals in research
PROJECT REPORT
Genomics & Biotechnology for Health
COOPERATION

EU
FP6 & FP7



Funding scheme	Number of projects	EC financial contribution (million €)
Sixth FP		
Integrated project	4	39.5
Specific Targeted Research Project	4	8.7
SME-Specific Targeted Research Project	5	13.0
Specific Support Action	8	2.1
Total	21	63.3
Seventh FP (First Call)	5	30.4 (planned)

AXLR8

Proposal Number:	241958
Project Type:	Coordination action
Starting date:	01.01.2010
Duration:	40 month

European Commission
DG Research and Technical Development
Health and Biotechnology
Alternative Testing Strategies
Brussels, Belgium

EU REACH Chemicals Legislation

Freie Universität Berlin

HUMANE SOCIETY INTERNATIONAL

CARDAM vito vision on technology

The CONSORTIUM

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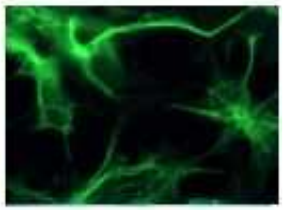
AXLR8

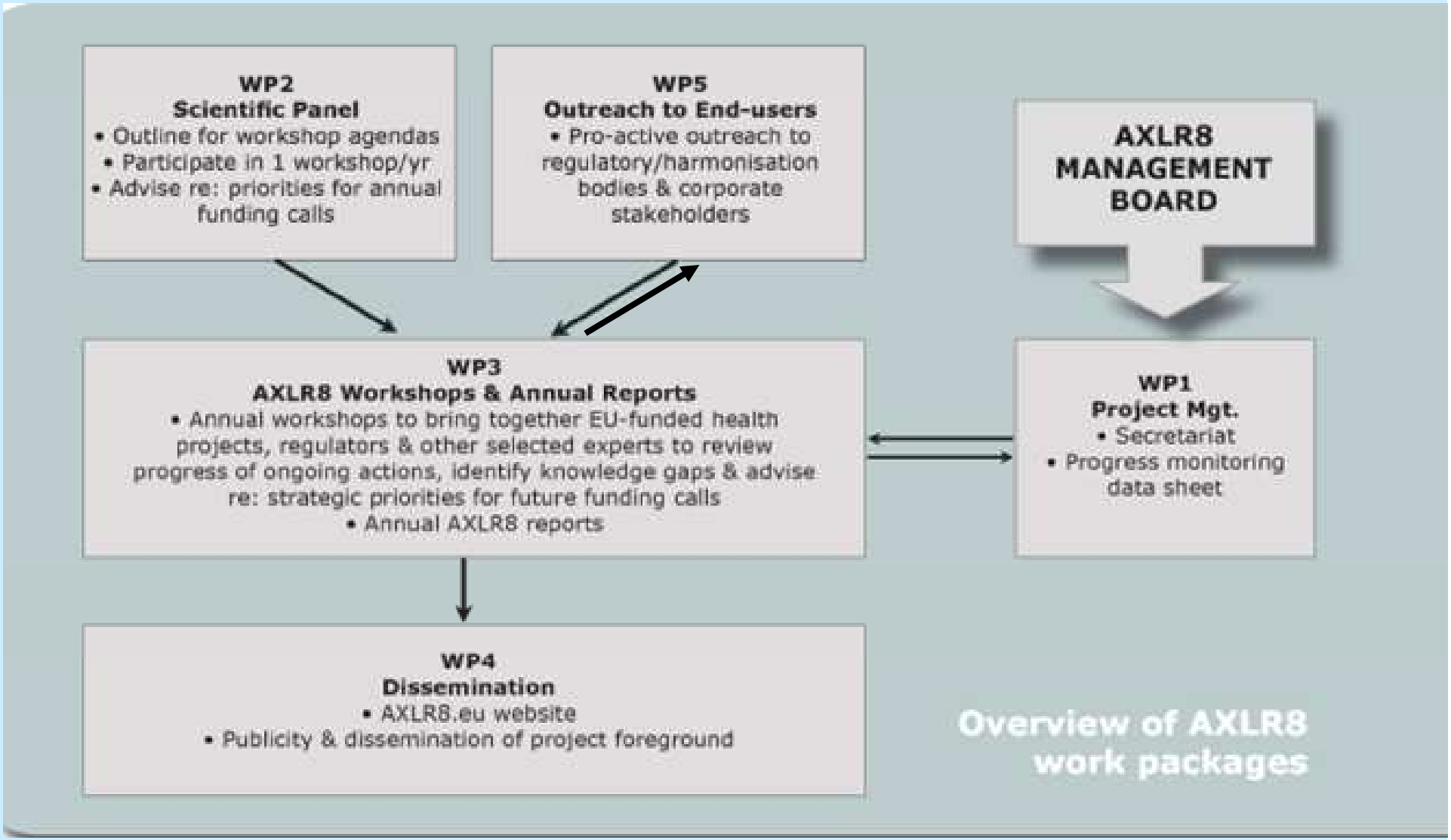
Accelerating the transition to a toxicity pathway-based paradigm for chemical safety assessment through internationally co-ordinated research and technology development



Freie Universität Berlin

The *AXLR8* project aims to support the transition to a toxicity pathway-based paradigm for quantitative risk assessment and will:

1) ***Organize a series of annual workshops*** to map research progress, gaps and needs in the FP6/FP7 program on alternative testing strategies.
2) ***Provide a range of tools and opportunities for enhanced interdisciplinary and international communication***, coordination and collaboration in order to maximise the impact of available resources.
3) ***Work to streamline regulatory acceptance*** procedures to provide for the uptake ***of validated 3Rs methods***, including a smooth transition to 21st century systems as they become available.
4) ***Produce annual progress reports on the state of the science*****,** including recommendations on priority research and funding targets, in order to ensure a prominent role for European science in this rapidly developing global research area.


WP2
Scientific Panel
• Outline for workshop agendas
• Participate in 1 workshop/yr
• Advise re: priorities for annual funding calls
WP5
Outreach to End-users
• Pro-active outreach to regulatory/harmonisation bodies & corporate stakeholders
AXLR8 MANAGEMENT BOARD
WP3
AXLR8 Workshops & Annual Reports
• Annual workshops to bring together EU-funded health projects, regulators & other selected experts to review progress of ongoing actions, identify knowledge gaps & advise re: strategic priorities for future funding calls
• Annual AXLR8 reports
WP1
Project Mgt.
• Secretariat
• Progress monitoring data sheet
WP4
Dissemination
• AXLR8.eu website
• Publicity & dissemination of project foreground
Overview of AXLR8 work packages

The AXLR8 Scientific Panel

Independent Members of the AXLR8 Scientific Panel

Name	*Affiliation*	*Country*
Nathalie Alépée	L'Oreal, cosmetic industry	FR
Patric Amcoff	OECD, Environment, Health and Safety Division	SWE & OECD
Jürgen Borlak	Fraunhofer Institute, research institute	DE
Steffen W. Ernst	AstraZeneca, Drug industry	SWE
Julia Fentem	Unilever, consumer products industry	UK
Ellen Fritsche	U. Duesseldorf, neurotoxicology	DE
Joanna Jaworska	Procter & Gamble, consumer product industry	BE
Robert Landsiedel	BASF, toxicology chemical industry	DE
Maurice Whelan	IHCP, JRC, EU research agency	IRL & EU
Mel Andersen	Hamner Institutes, research institute	USA
Robert Kavlock	EPA, US federal agency	USA
Hajime Kojima	JaCVAM, national validation centre	JAP

Representatives of FP6 & FP7 3Rs Projects on the AXLR8 Scientific Panel

Name	*Affiliation*	*Country*
Manuel Carrondo	IBET, VITROCELLOMICS	PT
Barry Hardy	OpenTox	CH
Juergen Hescheler	ESNATS	DE
Jos Kleinjans	carcinoGENOMICS	NL
Carl-Fredrik Mandenius	INVITROHEART, VITROCELLOMICS	SE
Michael Schwarz	ReProTect	DE
Flavia Zucco	LIINTOP	IT

A

AXLR8

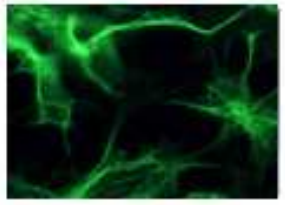
Accelerating the transition to a toxicity pathway-based paradigm for chemical safety assessment through internationally co-ordinated research and technology development

AXLR8-1 Workshop 2010

31 May – 2 June

Potsdam Griebnitzsee



AXLR8

ALTERNATIVE PROGRESS TESTING REPORT STRATEGIES 2010

Replacing, reducing and refining use of animals in research



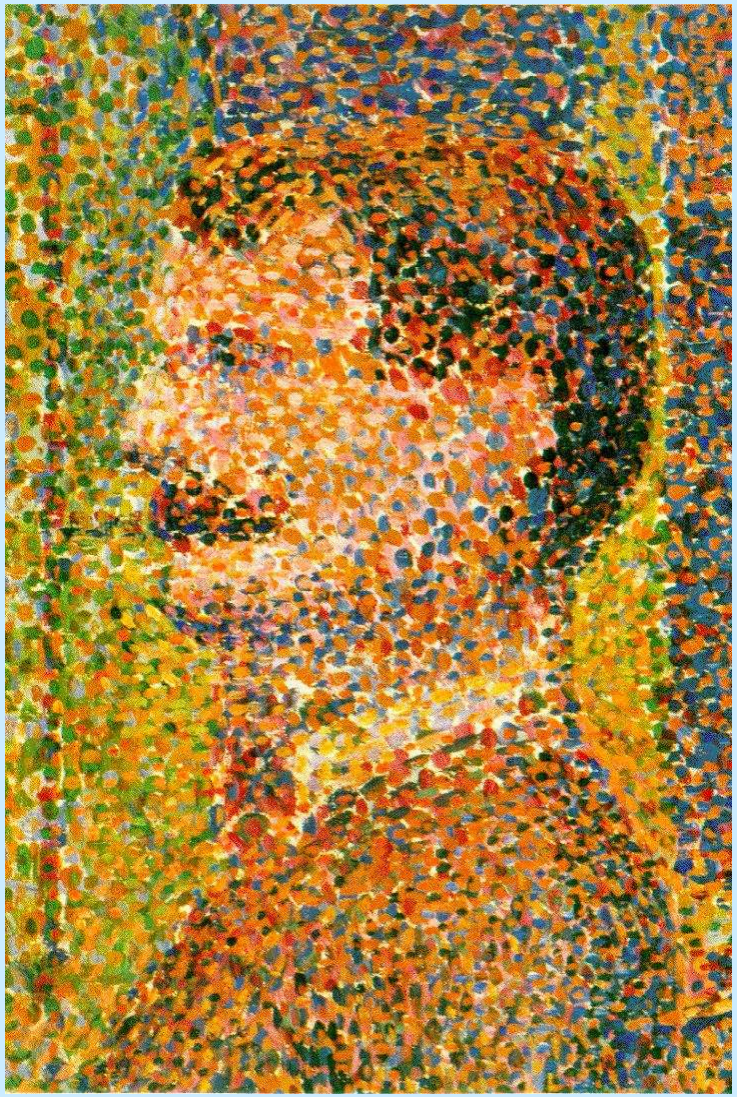
Health Biotechnology

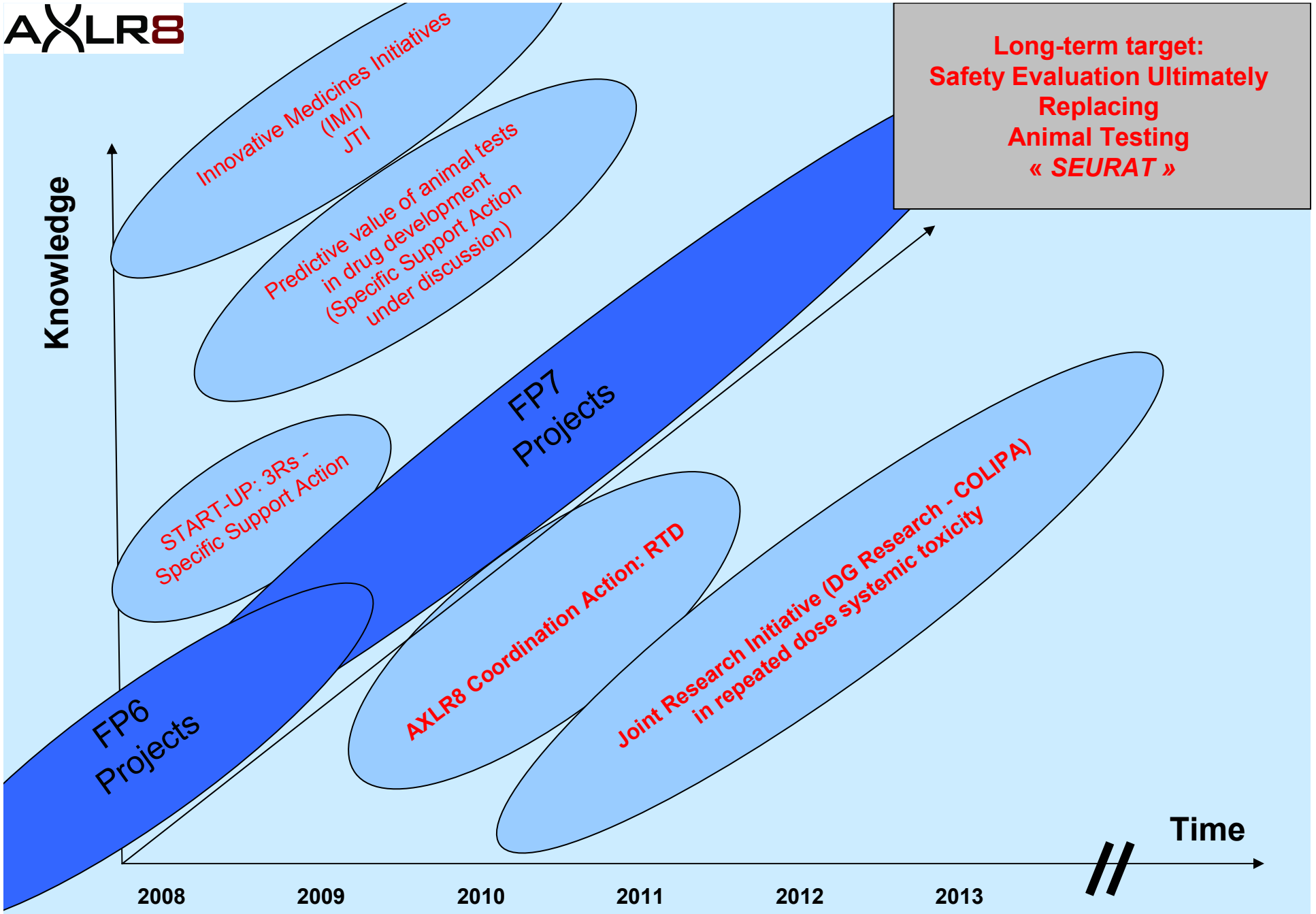
Grant Agreement N° 241958

Georges-Pierre SEURAT : detail from *La Parade* (1889) showing pointillism

EU Concept for Funding of Alternatives

<SEURAT>
Safety Evaluation Ultimately Replacing Animal Testing



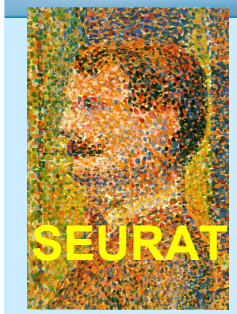

Long-term target:
Safety Evaluation Ultimately
Replacing
Animal Testing
« SEURAT »
Innovative Medicines Initiatives
(IMI)
JTI
Predictive value of animal tests
in drug development
(Specific Support Action
under discussion)
START-UP: 3Rs -
Specific Support Action
FP7
Projects
FP6
Projects
AXLR8 Coordination Action: RTD
Joint Research Initiative (DG Research - COLIPA)
in repeated dose systemic toxicity
Knowledge
Time
2008
2009
2010
2011
2012
2013

CONCLUSIONS 2008

The Participants agreed that there is considerable value in reconsidering the science base for regulatory testing in the field of repeated dose systemic toxicity.

The time is right to harness more effectively the substantial achievements that have been witnessed in biology and chemistry during the last 10 years.

Many discoveries and technological advances have the potential to impact substantially on the development of alternative approaches.



Towards replacement of repeated dose toxicity testing in human safety assessment

2011: EU & COLIPA funding for 6 years 50 Mio €

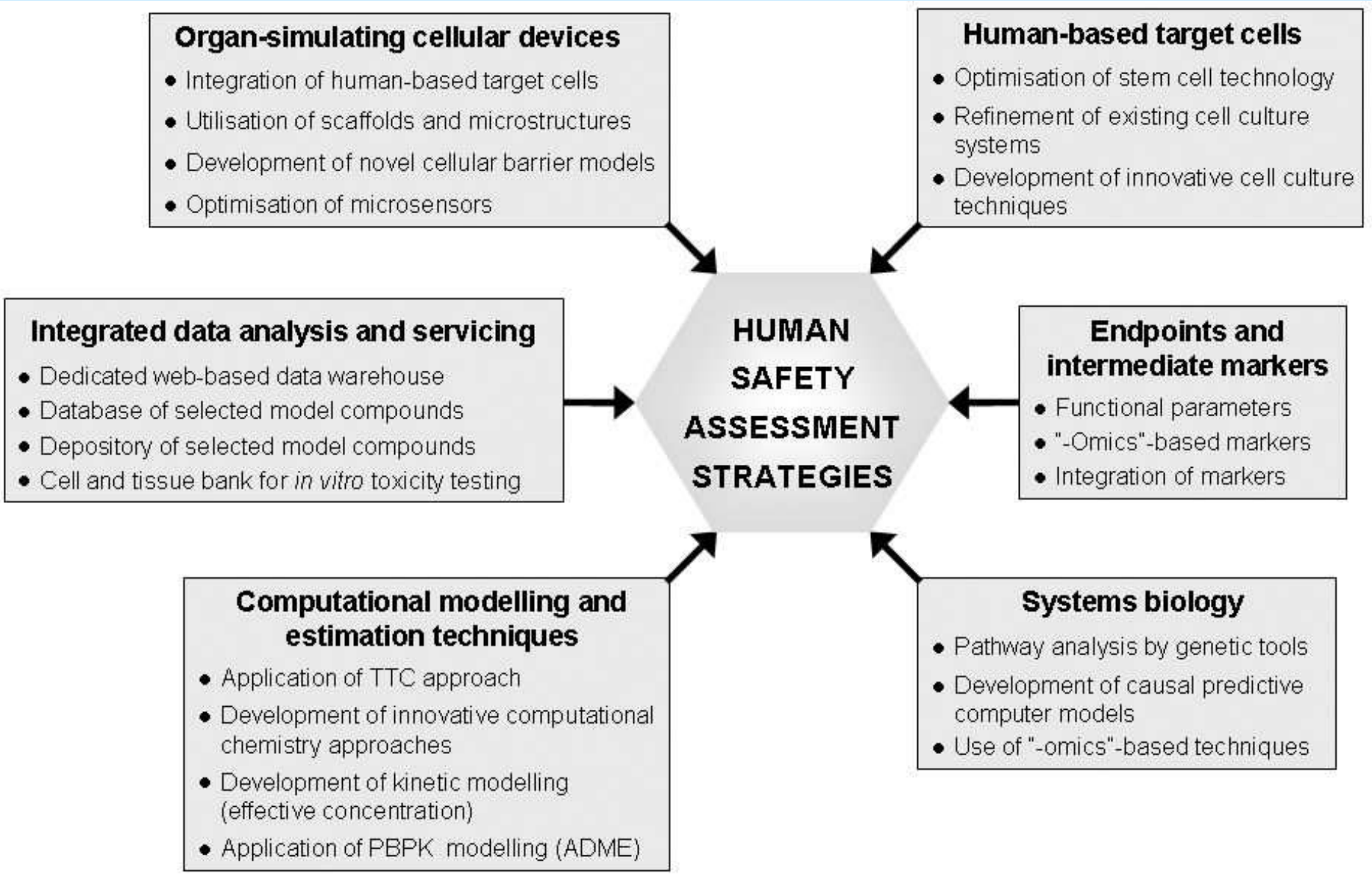
EU-Legislation, regulations and directives require urgently a phasing out of animal tests thus placing high pressure on research, validation and regulatory acceptance of non-animal alternatives.

The research plan for the first phase includes specific building blocks:

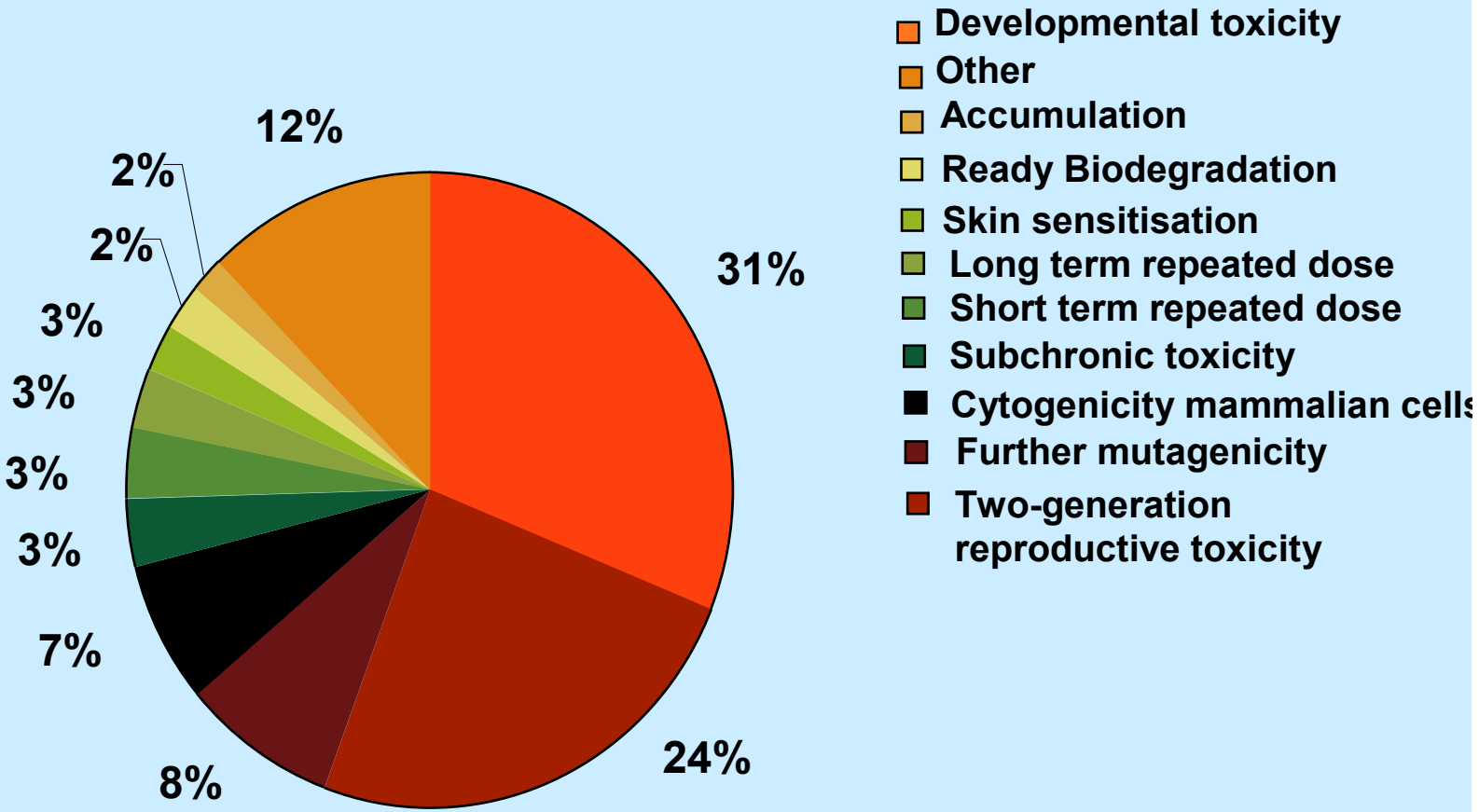
1. **Development and use of functional human-based target cells.**
2. **Construction of advanced organ-simulating devices.**
3. **Predictive endpoints and intermediate markers.**
4. **The development of biological models with emphasis on systems biology.**
5. **Computational modelling and estimation techniques.**
6. **Integrated data analysis and servicing.**

Safety Evaluation Ultimately Replacing Animal Testing

EU-COLIPA 2011



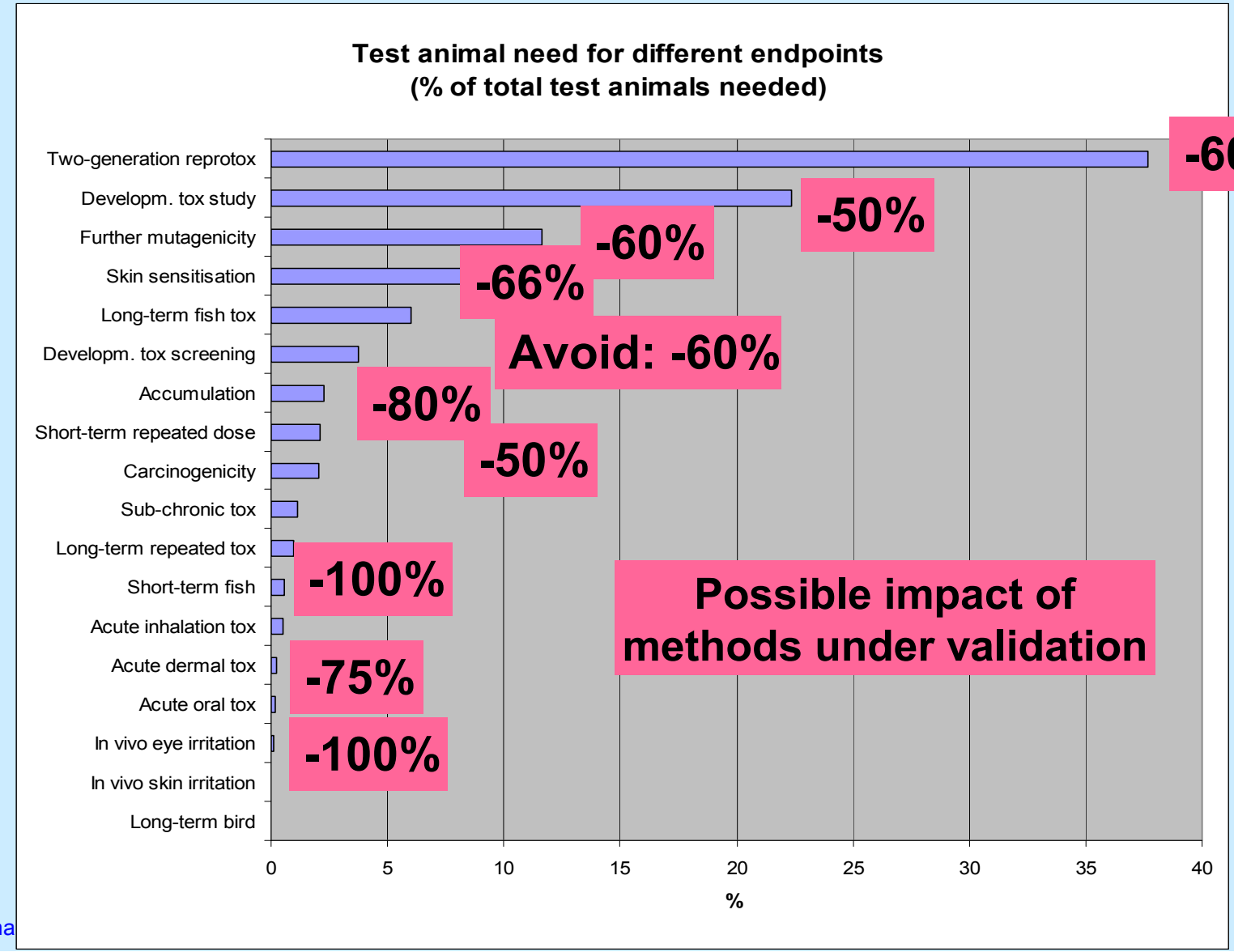
Estimated testing costs REACH



For reprotox and dev tox the costs may vary between about 700 million EURO and 1100 million EURO

Assessment of additional testing needs under REACH. Finn Pedersen, Jack de Bruijn, Sharon Munn & Kees van Leeuwen. EUR 20863 EN (2003) , http://ecb.jrc.it

Estimated test animal need for the different endpoints (EU Commission: van der Jagt et al. 2004)



Test Guidelines in Reproductive Toxicology

International Test Guidelines in Reproductive Toxicology

(EU, OECD, ICH)

Drugs (ICH)	Pesticides and Chemicals (REACH) (EU & OECD TGs)
Segment 1	teratology study (developmental tox study)
combined fertility & embryotoxicity study	
Segment 2	1-generation study
teratology study	
Segment 3	2-generation study
pre- and postnatal study	
	developmental neurotoxicity study

Why are we using different regulatory approaches in reproductive toxicity testing of drugs and chemicals ??

There are three approaches in regulatory toxicity testing depending on specific activity and exposure conditions:

1. **Drugs are designed to interact with specific receptors in humans**

2. **Pesticdes are designed to interact with receptors in the nervous system of insects, these receptors are also present in humans**

3. **Industrial chemicals (REACH) are not designed to interact with specific receptors in humans but to improve technical products**

This concept is not reflected in the current testing requirements in developmental toxicity testing:

1. The testing requirements for drugs are less stringent than for pesticides.
2. Industrial chemicals are less toxic than pesticides and drugs.
3. Current REACH testing requirements for industrial chemicals are driven by testing requirements for pesticides, in particular the **2-generation study** (***which is not required for drugs***).

Is this concept still valid ??

The way forward ➔ critical evaluation of reprotox data in industry and regulatory agencies

1. Can reproductive toxicity testing of chemicals be reduced to the current requirements for drugs ?
2. For industrial chemicals industry and regulators must provide evidence that 2-gen studies are contributing data (NOEL/LOEL) that are essential for regulatory decisions.

ATLA 34, 365–366, 2006

Editorial

REACH Testing Requirements Must Not be Driven by Reproductive Toxicity Testing in Animals

Horst Spielmann and Richard Vogel[4]
National Centre for Documentation and Evaluation of Alternative Methods to Animal Experiments (ZEBET), Federal Institute for Risk Assessment (BfR), Berlin, Germany

3. It must be borne in mind that the chemicals used in drugs and pesticides are designed to interact with active molecules at the cellular level in the human body and in the pest species, respectively. In contrast, industrial chemicals are designed to improve the functions of chemical products for quite different purposes, often as a result of their physical properties, rather than their chemical reactivity. Thus, in contrast to drugs and pesticides, a large proportion of industrial chemicals are not toxic to reproduction.

5. There is no substantial evidence to prove a correlation between the adverse effects observed in two-generation studies in rodents and human reproduction and fertility.

6. When the two-generation study was introduced as an OECD Test Guideline in 1982, the test was not formally validated with respect to its relevance for human reproduction and fertility.

A retrospective analysis of the two-generation study: What is the added value of the second generation?

Gemma Janer *, Betty C. Hakkert, Wout Slob, Theo Vermeire, Aldert H. Piersma

National Institute of Public Health and the Environment (RIVM), PO Box 1, 3720 BA Bilthoven, The Netherlands

Received 16 February 2007; received in revised form 10 April 2007; accepted 25 April 2007
Available online 6 May 2007

Reproductive Toxicology 24 (2007) 97–102

- **176 multi generation studies for 146 chemicals**
- **OECD 415 – one generation study**
- **OECD 416 – two generation study**
- **NOEL derived for reproductive endpoints**

Peer-reviewed national or international data sources used
EU Risk Assessment Reports for Existing Substances
OECD Screening Information Dataset for High Production Volume Substances
Joint Meeting on Pesticide Residues
Environmental Health Criteria Monographs
Concise International Chemical Assessments Documents
US Agency for Toxic Substances and Disease Registry
US National Toxicology Program-Center for the Evaluation of Risks to Human Reproduction
Canadian Pest Management Regulatory Agency
Health Council of the Netherlands
German Advisory Committee on Existing Chemicals of Environmental Relevance
California EPA Evaluation Reports

Janer et al., Reproductive Toxicol 24, 97-102, 2007

CONCLUSIONS

The second generation in the two-generation studies affected neither the overall NOAEL nor the critical effect. Therefore, it had no impact on the resulting risk assessment, nor on classification and labeling.
However, several substances did show an increased sensitivity of the F1 adults in comparison to the P0.

These results support the proposal of replacing the current 2-generation study by a 1-generation study with a more extensive assessment of parameters at F1 adulthood.

►US-EPA retrospective data analysis supports NL data analysis

OECD since 2007

OECD TEST GUIDELINES PROGRAMME:
Proposal of a new/updated Test Guideline in 2007

Standard Project Submission Form - SPSF

PROJECT TITLE

Extended F1 Generation Reproductive Toxicity Study
(EOGRTS = OECD *DRAFT* TG 415)

SUBMITTED BY

United States (Dr. Jerry Smrchek, U.S. National Coordinator)
Germany (Dr. Petra Greiner, DE National Coordinator)
The Netherlands (Betty Hakkert, NL National Coordinator)

17 November 2010

OECD GUIDELINE FOR THE TESTING OF CHEMICALS

Extended One-Generation Reproductive Toxicity Study

ReProTect

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

Contract number:	LSHB-CT-2004-503257
Project type:	Integrated Project (FP6)
EC contribution:	€ 9 100 000
Starting date:	1 July 2004
Duration:	66 months

Website: http://www.reprotect.eu

FP6

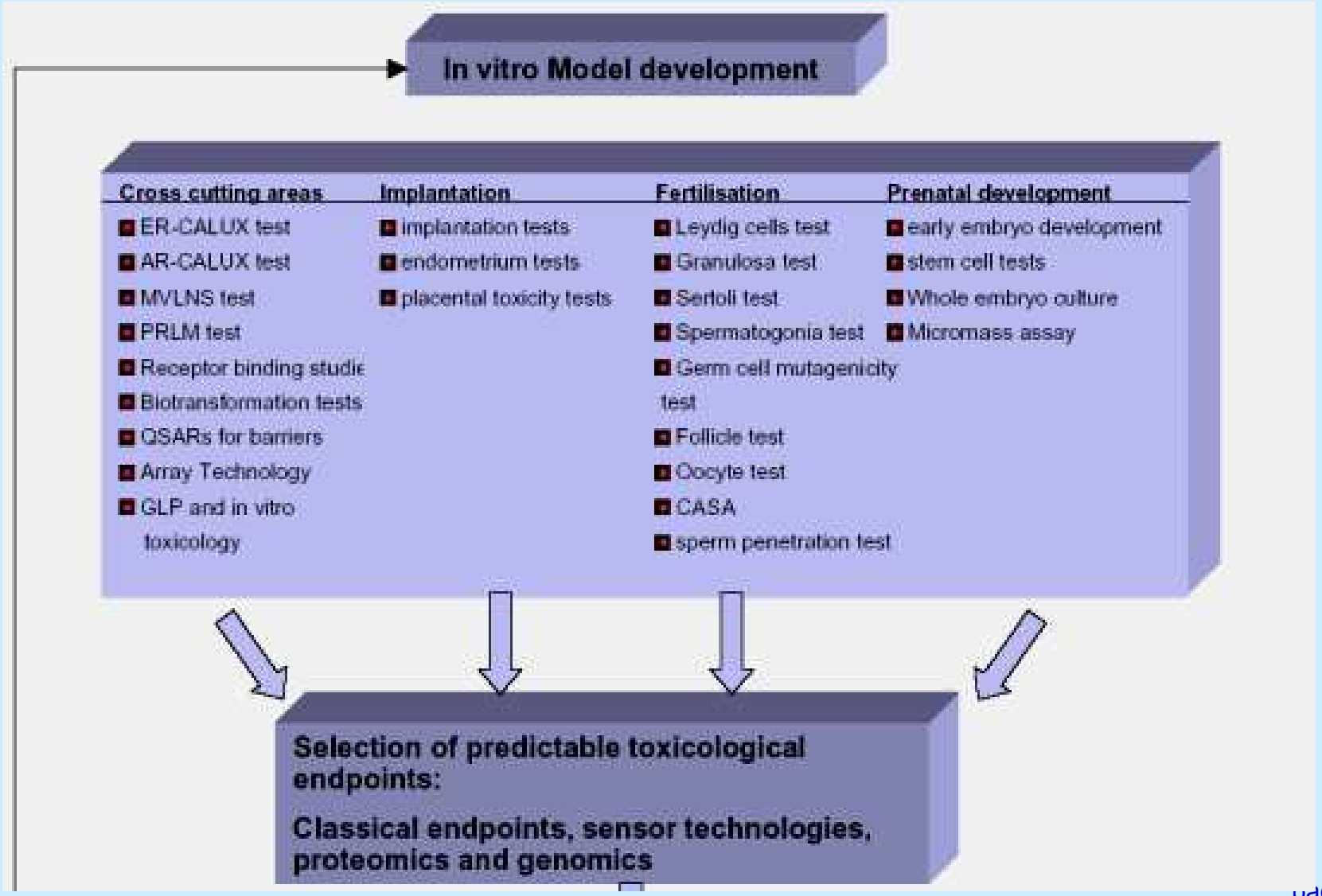
ESNATS

Embryonic stem cell-based novel alternative testing strategies

Grant agreement number:	HEALTH-F5-2008-201619
Project type:	Collaborative Project (Large-scale integrating project) (FP7)
EC contribution:	€ 11 895 577
Starting date:	1 April 2008
Duration:	60 months

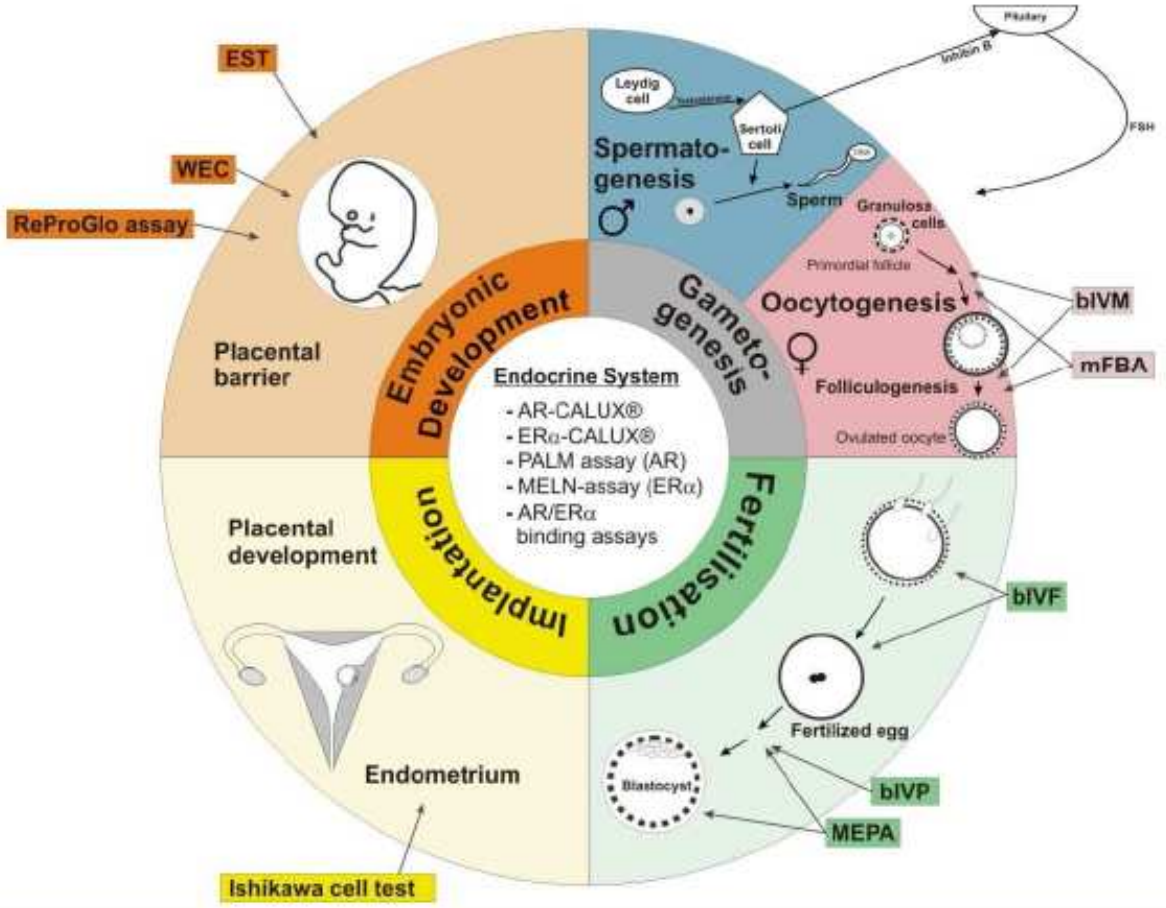
Website: http://www.esnats.eu

FP7


In vitro Model development
Cross cutting areas
ER-CALUX test
AR-CALUX test
MVLNS test
PRLM test
Receptor binding studie
Biotransformation tests
QSARs for barriers
Array Technology
GLP and in vitro toxicology
Implantation
implantation tests
endometrium tests
placental toxicity tests
Fertilisation
Leydig cells test
Granulosa test
Sertoli test
Spermatogonia test
Germ cell mutagenicity test
Follicle test
Oocyte test
CASA
sperm penetration test
Prenatal development
early embryo development
stem cell tests
Whole embryo culture
Micromass assay
Selection of predictable toxicological endpoints:
Classical endpoints, sensor technologies, proteomics and genomics

Partners and tests in the Feasibility Study

Michael Schwarz
Giovanna Lazzari
Bart van der Burg
Aldert Piersma
Alexius Freyberger
Rita Cortvrindt
Andrea Seiler
Witters Hilda
Wolfgang Schaefer
Stefano Lorenzetti
Axel Themmen



The ReProTect Feasibility Study

Reproductive Toxicology 30 (2010) 200–218

Effect:
Positive (red)
or negative (green)

√ Correctly predicted

(√) Partly predicted

x Not correctly predicted

Chemical	Female fertility	Male fertility	Developmental toxicity
1	√	√	√
2	√	√	√
3	x	x	√
4	√	√	√
5	√	√	√
6	√	√	√
7	√	?	(√)
8	√	x	√
9	√	√	(√)
10	√	√	√

EST 10x OK

nature protocols

Andrea Seiler & Horst Spielmann, 2011

The validated embryonic stem cell test to predict embryotoxicity *in vitro*

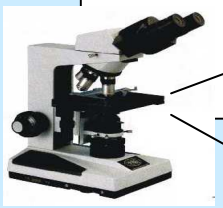
mouse ES cell line D3

differentiated cells

Endpoint 1:

inhibition of differentiation

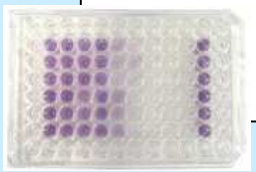
ES cells



Endpoint 2:

cytotoxic effects

ES cells



Endpoint 3:

cytotoxic effects

3T3 cells

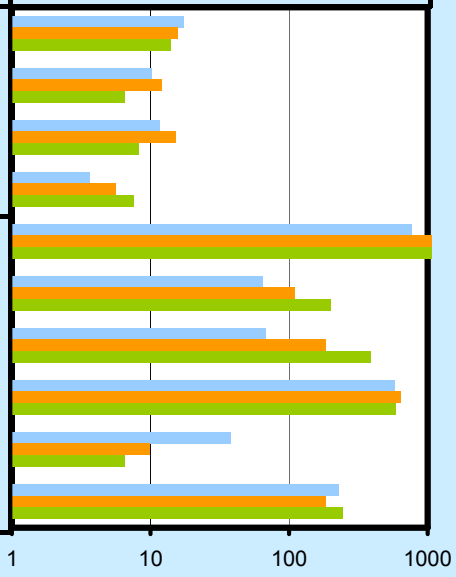
Endpoints: assessment from concentration response curves

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

Comparison of the results obtained in the validated EST

	ID_{50} (myocardial differentiation)			$IC_{50}D3$ (Cytotoxic effects on ES cells)			$IC_{50}3T3$ (Cytotoxic effects on 3T3 fibroblasts)			in vivo classi-fication (Daston et al.)
		confidence interval			confidence interval			confidence interval		
Chemical	ID_{50}	Lower bound	Upper bound	$IC_{50}D3$	Lower bound	Upper bound	$IC_{50}3T3$	Lower bound	Upper bound	
Dino	17.3	14.6	20.5	15.8	13.3	18.9	13.9	9.83	19.7	3
Ochra	10.2	9.64	10.8	12.1	8.71	16.7	6.54	5.96	7.17	1
Nitrofen	11.7	11.5	11.9	15.3			8.18	7.48	8.95	1
Lova	3.65	3.6	3.7	5.61	5.19	6.06	7.56	6.11	9.37	2
β-APN	765	763	767	1280	995	1640	1070	846	1350	3
Meto	64.4	63.9	64.9	109	96.3	123	200	69.8	574	4
Doxy	68.3	65.3	71.4	184	66.8	509	387	121	1240	4
D-Penic	581	514	657	634	506	794	589	544	638	1
MAM	37.7	34.2	41.6	9.9	4.66	21	6.54	5.38	7.96	1
Warf	228	194	268	184	165	206	245	230	260	2

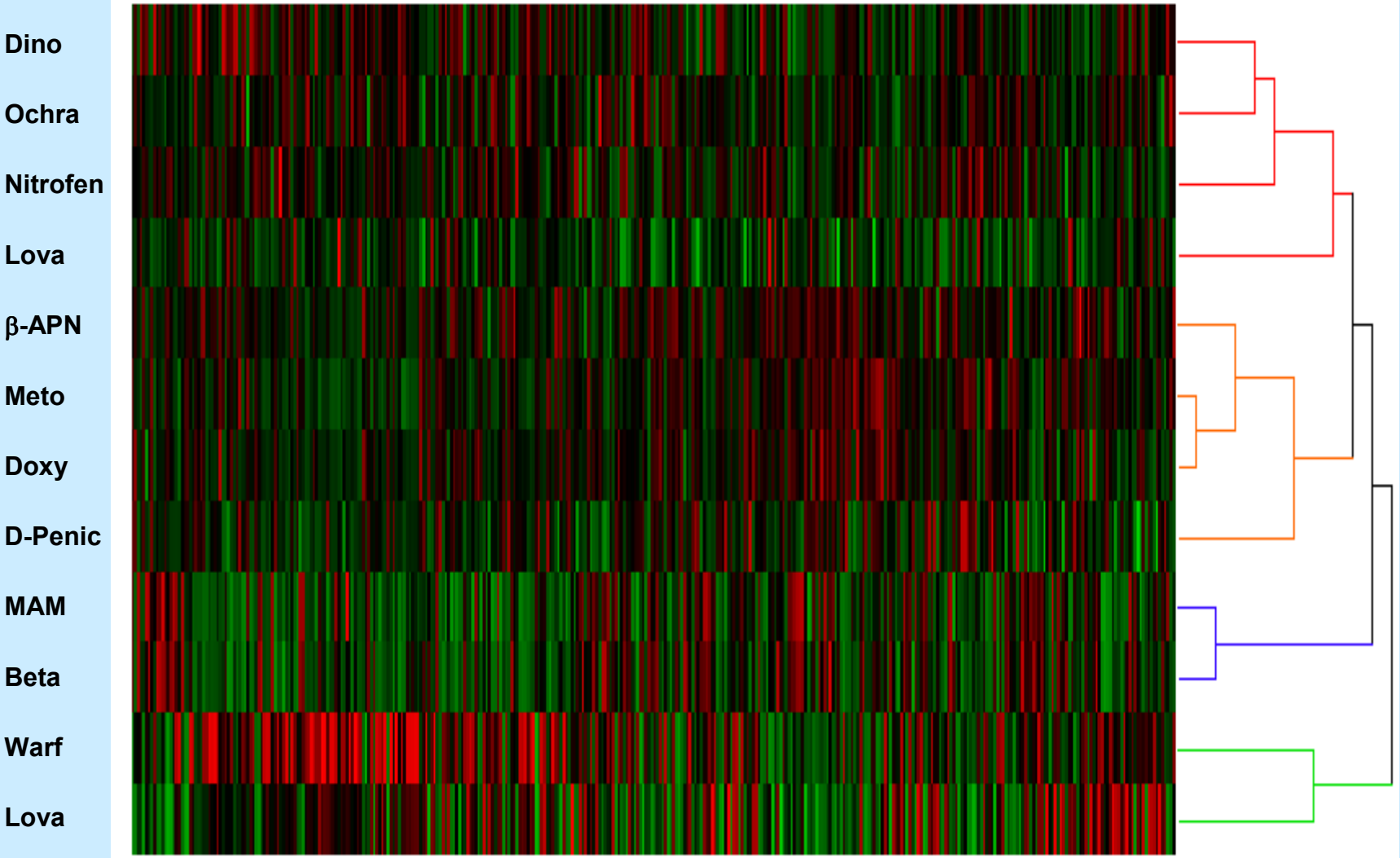
Synopsis of ID_{50}, $IC_{50}D3$, $IC_{50}3T3$



Protein biomarkers for in vitro testing of embryotoxicity in the mEST
Andre Schrattenholz (Proteosys) et al. J. Proteome Res. 2010

Protein biomarkers for in vitro testing of embryotoxicity in the mEST

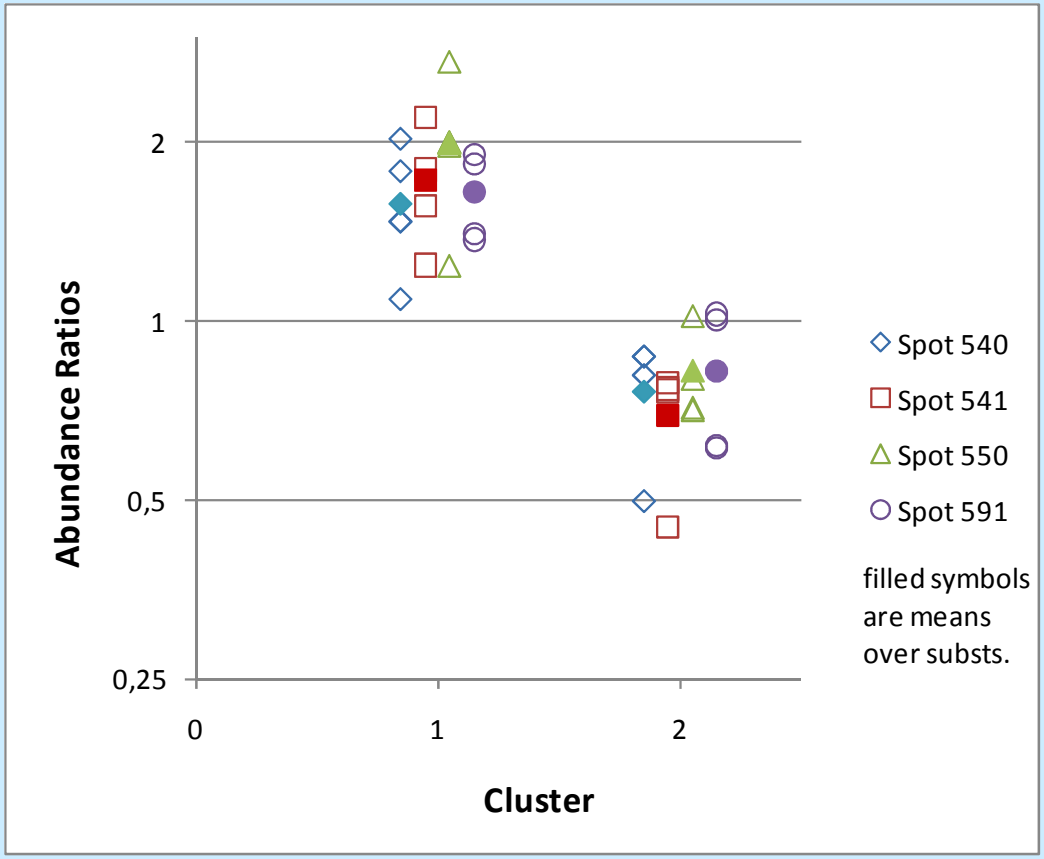
380 differential proteins were found and identified by MALDI-TOF mass spectrometry



Andre Schrattenholz (Proteosys) et al. J. Proteome Res. 2010

Protein biomarkers for in vitro testing of embryotoxicity in the mEST

Andre Schrattenholz (Proteosys) et al, J Proteome Res. 2010



G3BP = Ras-GTPase-activating SH3-domain binding protein

Abundance ratios of G3BP isoforms in protein spots 540, 541, 550, and 591 for substances in clusters 1 and 2 (abscissa). It turns out that all isoforms respond very similar to all substances from the same cluster. Distinctly different abundance ratios exist for substances from different clusters

Reproductive Toxicology 30 (2010) 121–130

Unexpected common mechanistic pathways for embryotoxicity of warfarin and lovastatin

Karlfried Groebe[a], Katrin Hayess[b], Martina Klemm-Manns[a], Gerhard Schwall[a], Woijciech Wozny[a], Margino Steemans[c], Annelieke K. Peters[c], Chaturvedala Sastri[a], Petra Jaeckel[a], Werner Stegmann[a], Helmut Zengerling[a], Rainer Schöpf[a], Slobodan Poznanovic[a], Tina C. Stummann[d], Andrea Seiler[b], Horst Spielmann[b], André Schrattenholz[a,*]

[a] *ProteoSys AG, Carl-Zeiss-Str. 51, D-55129 Mainz, Germany*
[b] *Federal Institute for Risk Assessment, Center for Alternative Methods to Animal Experiments – ZEBET Diedersdorfer Weg 1, 12277 Berlin, Germany*
[c] *Johnson & Johnson PRD, A Division of Janssen Pharmaceutical, 2340 Beerse, Belgium*
[d] *ECVAM (IHCP, JRC) Via Fermi, 121020 Ispra, Italy*

	ID_{50} (myocardial differentiation)			$IC_{50}D3$ (Cytotoxic effects on ES cells)			$IC_{50}3T3$ (Cytotoxic effects on 3T3 fibroblasts)			in vivo classification (Daston et al.)
		confidence interval			confidence interval			confidence interval		
Chemical	ID_{50}	Lower bound	Upper bound	$IC_{50}D3$	Lower bound	Upper bound	$IC_{50}3T3$	Lower bound	Upper bound	
Lova	3,65	3,6	3,7	5,61	5,19	6,06	7,56	6,11	9,37	2
Warf	228	194	268	184	165	206	245	230	260	2

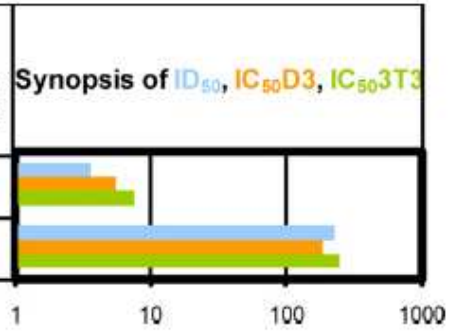
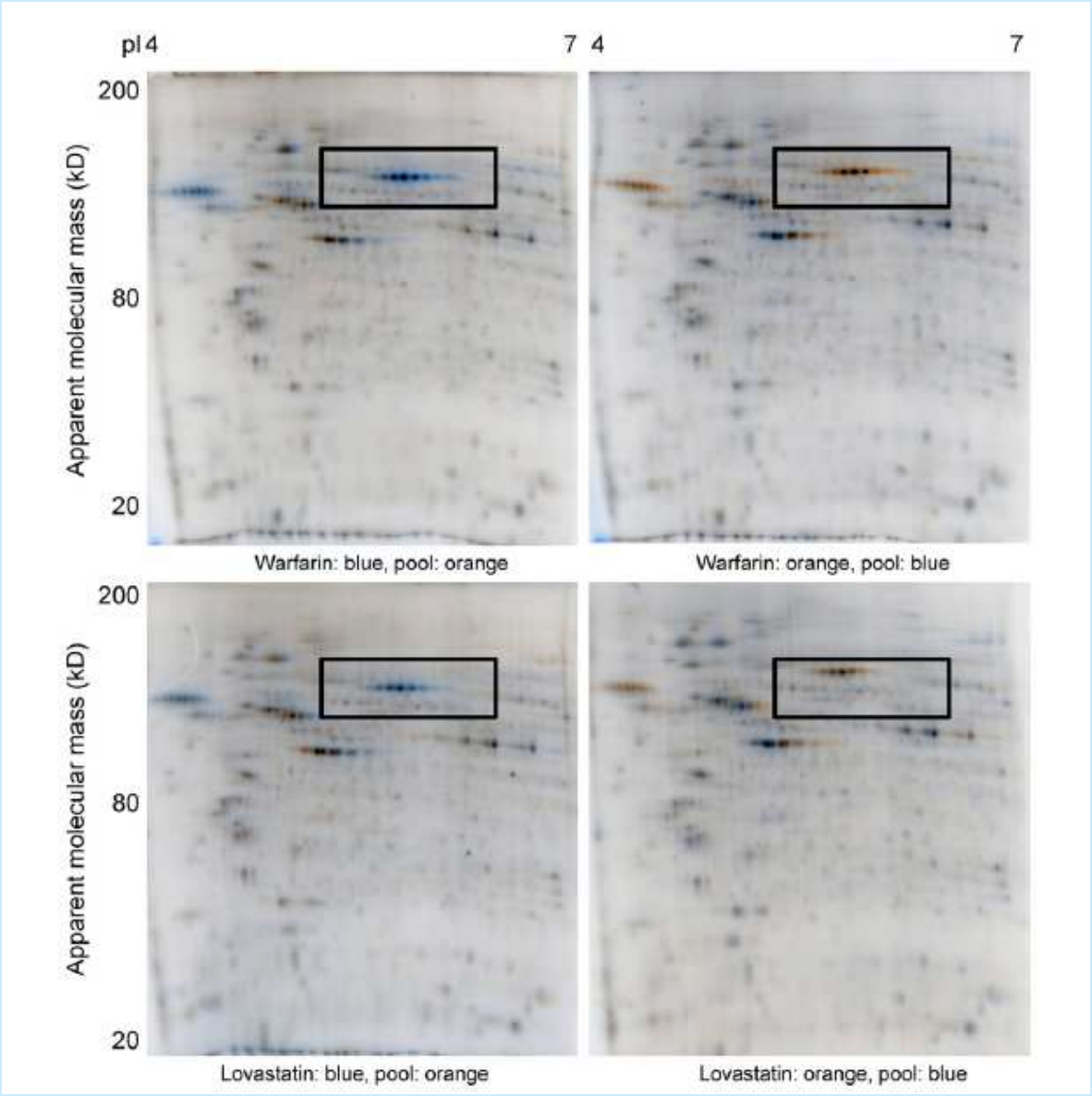
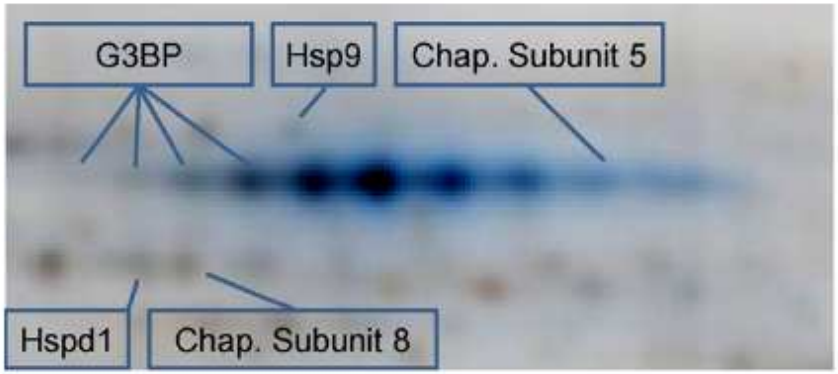
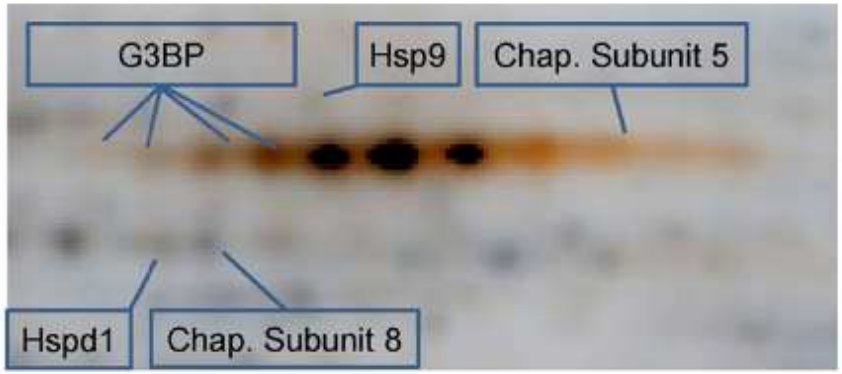


Fig. 2. Comparison of the results of the validated EST. ID_{50}, $IC_{50}D3$ and $IC_{50}3T3$ values are denoted together with the lower and the upper boundary of the respective confidence interval. The ID_{50} and IC_{50} values were confirmed in an independent second run (data not shown). In the synopsis column the values for 50% inhibition of cardiomyocyte differentiation (ID50) as well as for 50% inhibition of growth of mESC ($IC_{50}D3$) and 3T3 fibroblasts ($IC_{50}3T3$) are shown.

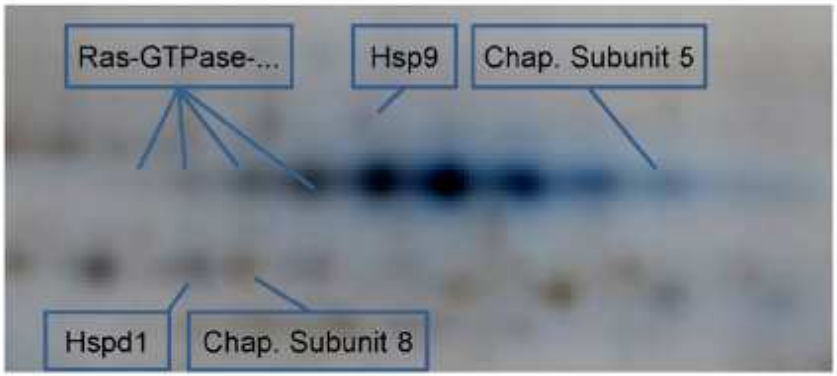

pI 4
7
4
7
200
80
20
Apparent molecular mass (kD)
Warfarin: blue, pool: orange
Warfarin: orange, pool: blue
200
80
20
Apparent molecular mass (kD)
Lovastatin: blue, pool: orange
Lovastatin: orange, pool: blue



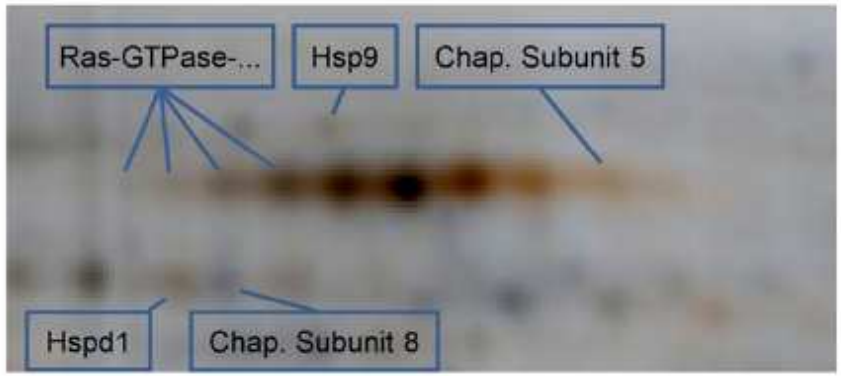
Warfarin: blue, pool: orange



Warfarin: orange, pool: blue



Lovastatin: blue, pool: orange



Lovastatin: orange, pool: blue

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

Vol 247, 2010, 18-27

journal homepage: www.elsevier.com/locate/ytaap

Predicting human developmental toxicity of pharmaceuticals using human embryonic stem cells and metabolomics

Paul R. West [a,*], April M. Weir [a], Alan M. Smith [a], Elizabeth L.R. Donley [a], Gabriela G. Cezar [a,b]

[a] Stemina Biomarker Discovery, Inc., 504 S. Rosa Rd., Suite 150, Madison, WI 53719, USA
[b] University of Wisconsin-Madison, Department of Animal Sciences, 1675 Observatory Drive, Madison WI 53706, USA

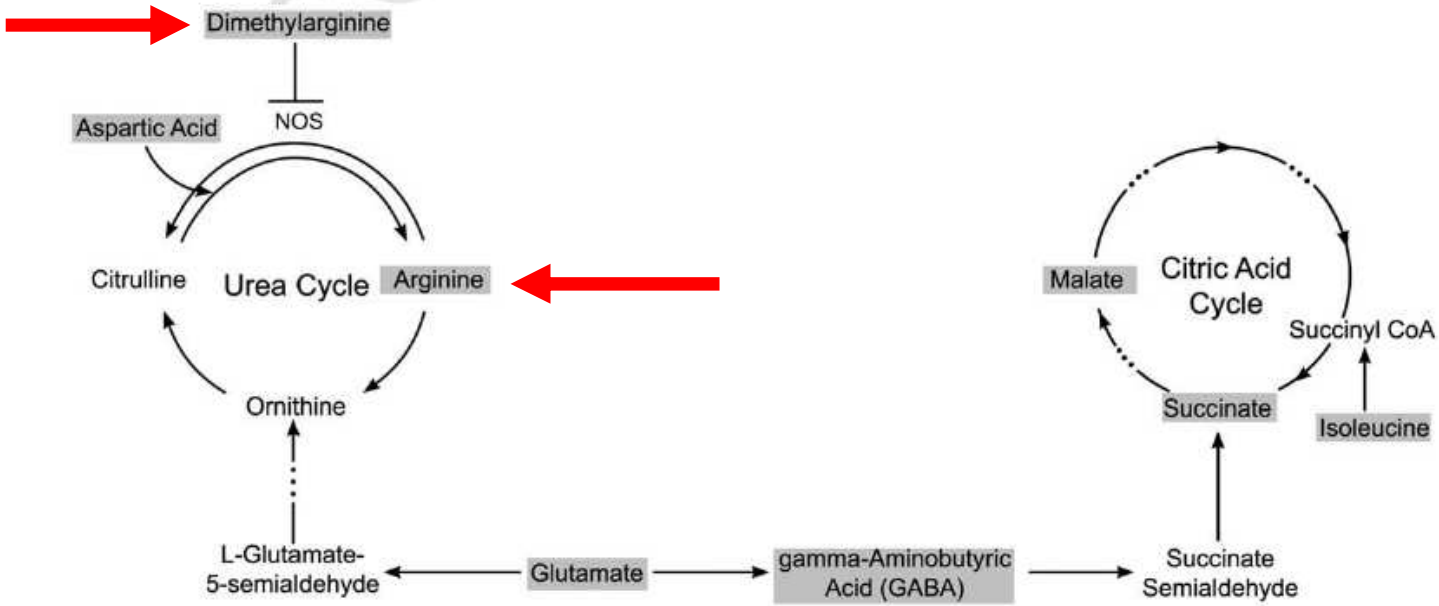


Fig. 3. Illustration of the metabolic pathway relationships between the metabolites found in this study.

Table 5
Selected fold change ratios for arginine and asymmetric dimethylarginine (ADMA). EICs for these compounds were integrated, then the fold change of the resulting areas for controls vs. dosed were compared. Smaller fold change ratios (between 0.9 and 1.1) show a good correlation with non-teratogens, while greater changes (<0.9 and >1.1) correlate with teratogens. There are no false negatives for teratogenicity resulting from these metrics and only ascorbic acid and caffeine are false positives.

Stemina classification	Compound	Arg fold change/ADMA fold change	Arg/ADMA prediction
Non-teratogens	Ascorbic acid	1.28	Ter
	Aspirin	1.07	Non
	Caffeine	1.33	Ter
	Doxylamine (Blind 2)	0.97	Non
	Isoniazid	0.94	Non
	Levothyroxine	1.03	Non
	Penicillin G	0.96	Non
	Folic acid	1.08	Non
	Retinol (Blind 1)	1.03	Non
	Thiamine (Blind 8)	1.00	Non
Teratogens	5-Fluorouracil	43.93	Ter
	Methotrexate	2.54	Ter
	Accutane (Blind 6)	0.55	Ter
	Amiodarone (Blind 3)	1.64	Ter
	Busulfan	1.12	Ter
	Carbamazepine (Blind 5)	1.12	Ter
	Cyclophosphamide (Blind 7)	1.56	Ter
	Cytosine arabinoside	67.01	Ter
	Hydroxyurea	2.52	Ter
	Retinoic acid	0.48	Ter
	Rifampicin (Blind 4)	0.81	Ter
	Thalidomide	0.85	Ter
	Valproic acid	2.11	Ter

Contents lists available at ScienceDirect

Reproductive Toxicology

journal homepage: www.elsevier.com/locate/reprotox

Metabolic activation capacity by primary hepatocytes expands the applicability of the embryonic stem cell test as alternative to experimental animal testing

Michael Hettwer[a,*], Marcos A. Reis-Fernandes[a], Marcus Iken[b], Michael Ott[b], Pablo Steinberg[a], Heinz Nau[a]

[a] Institute for Food Toxicology and Analytical Chemistry, University of Veterinary Medicine Hannover, Bischofsholer Damm 15, 30173 Hannover, Germany
[b] Centre of Experimental and Clinical Research: Twincore, Gastroenterology, Hepatology and Endocrinology, Hannover Medical School, 30625 Hannover, Germany

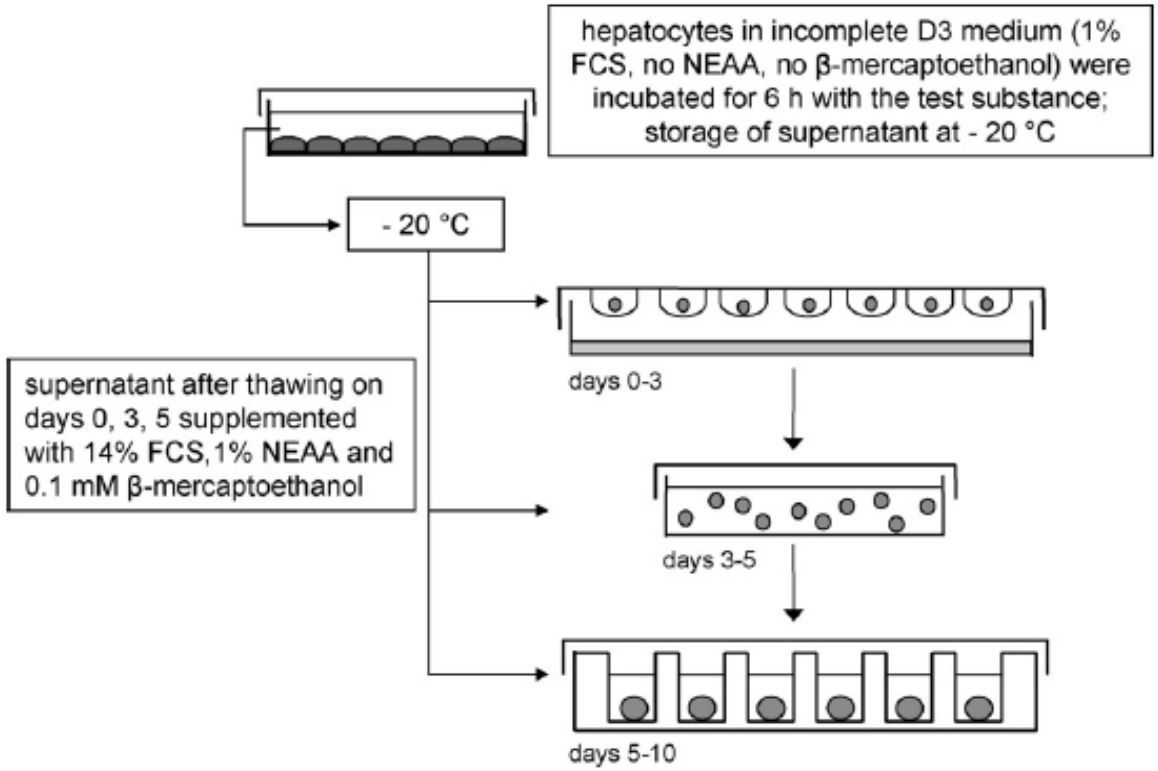


Fig. 1. Combination of the hepatocyte incubation step with the EST.

mEST at Pfizer

Original Article

Assessment of the Embryonic Stem Cell Test and application and use in the pharmaceutical industry

Jennifer A. Paquette *, Steven W. Kumpf, Randal D. Streck, Jason J. Thomson, Robert E. Chapin, Donald B. Stedman
Pfizer Global Research & Development, Global DART, Groton/New London Laboratories, Pfizer Inc., Groton, Connecticut
email: Jennifer A. Paquette (jennifer.a.paquette@pfizer.com)

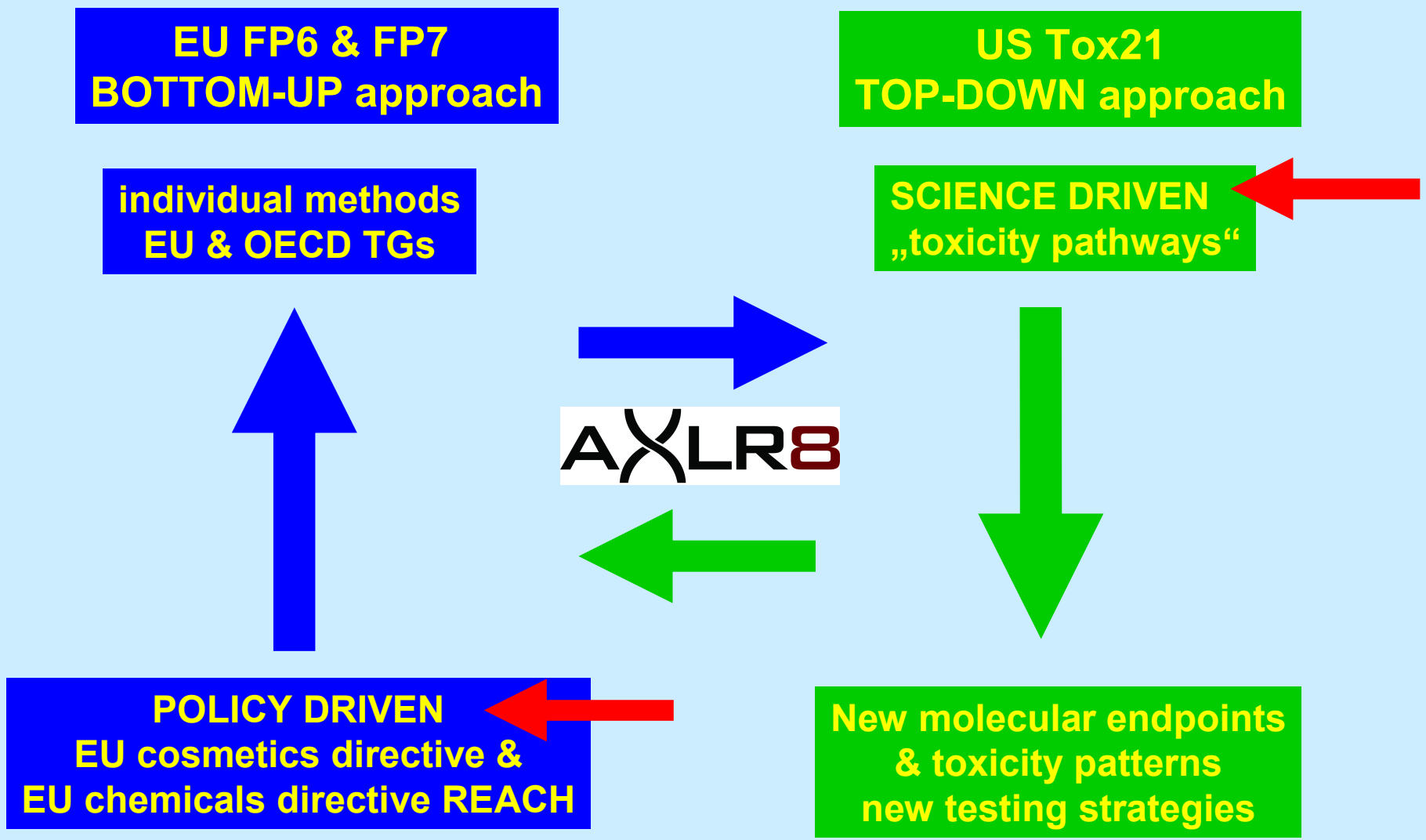
Birth Defects Research Part B: Developmental and Reproductive Toxicology **2008**

Collectively, our study confirms that the overall performance of the mEST was generally good and that this assay can be used in commercial settings to aid in generating compound-development decisions.

Possible improvements or refinements that might improve identification of developmental toxicity include the use of transcriptional expression profiling of the differentiating embryoid bodies, a possible approach already stated by Buesen et al. ([2004]).

Future work will focus on testing more true moderate- and high-risk compounds and then working to re-derive the predictive linear discriminant functions to achieve a better separation of low-risk from moderate-risk compounds, an issue already foreseen by ECVAM and Spielmann et al. ([2006]).

EU and US approaches to advanced toxicity testing



ZEBET's Achievements: OECD Test Guidelines (TG) & Guidance Documents (GD)

1. Complete replacement of the animal experiment

TG 428: Skin Absorption: In vitro Method

TG 430: In vitro Skin Corrosion: Transcutaneous Electrical Resistance (TER)

TG 431: In vitro Skin Corrosion: Human Skin Model Test

TG 432: In vitro 3T3 NRU Phototoxicity Test

TG 437: Bovine Corneal Opacity and Permeability Test Method for Identifying Ocular Corrosives and Severe Irritants

TG 438: Isolated Chicken Eye Test Method for Identifying Ocular Corrosives and Severe Irritants

TG 439: In vitro Skin Irritation: Reconstructed Human Epidermis (RhE) Test Method

2. Reduction in the number of animals and stress of the laboratory animals

TG 415b: Extended One-Generation Reproductive Toxicity Study

TG 420: Acute Oral Toxicity — Fixed Dose Procedure

TG 423: Acute Oral toxicity — Acute Toxic Class Method

TG 425: Acute Oral Toxicity: Up-and-Down Procedure

TG 429: Skin Sensitisation: Local Lymph Node Assay

TG 436: Acute Inhalation Toxicity — Acute Toxic Class Method

GD 14: Detailed Review Document on Classification Systems for Eye Irritation/Corrosion in OECD Member Countries

GD 16: Detailed Review Document on Classification Systems for Skin Irritation/Corrosion in OECD Member Countries

GD 19: Guidance Document on the Recognition, Assessment and Use of Clinical Signs as Humane Endpoints

GD 24: Guidance Document on Acute Oral Toxicity Testing

GD 28: Guidance Document for the Conduct of Skin Absorption Studies

GD 34: Guidance Document on the Validation and International Acceptance of New or Updated Test Methods for Hazard Assessment

GD 39: Guidance Document on Acute Inhalation Toxicity Testing

GD 69: Guidance Document on the Validation of (Quantitative) Structure-Activity Relationship [(Q)SAR] Models

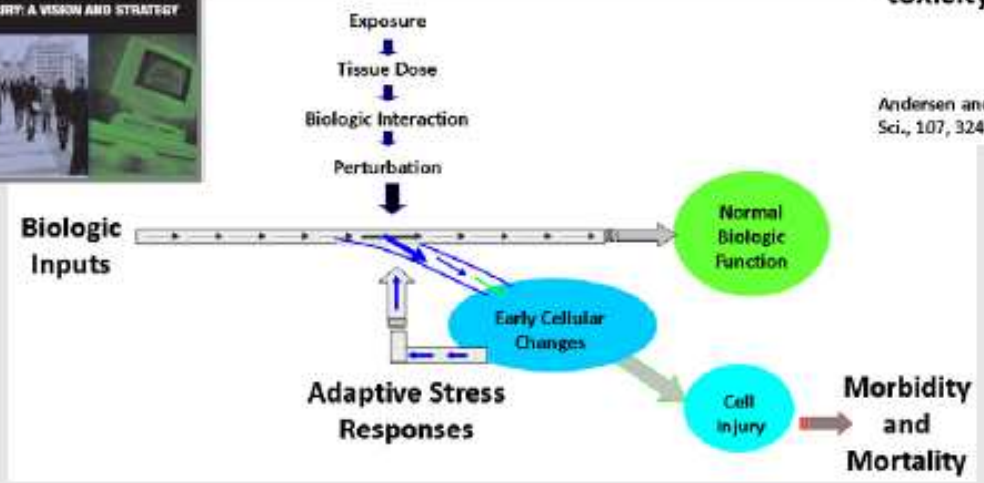
GD 105: Report on Biostatistical Performance Assessment of the Draft TG 436 Acute Toxic Class Testing Method for Acute Inhalation Toxicity

Human Toxicology Project Consortium

A multi-stakeholder coalition
dedicated to accelerating the global implementation
of 21st Century Toxicology in general,
and the NRC vision in particular,
into risk assessment,
to better safeguard human health and
hasten the replacement of animal use in toxicology.

HUMAN TOXICOLOGY PROJECT **CONSORTIUM**

U.S. National Research Council (2007)



... a not-so-distant future where all routine toxicity testing will be conducted in human cells or cell lines *in vitro* by evaluating perturbations of cellular responses in a suite of toxicity pathway assays.

Andersen and Krewski (2009). Toxicity Testing in the 21st Century: Bringing the Vision to Life. Tox. Sci., 107, 324-330.

"TT21C"

Likely benefits:

- More relevant scientific foundation for human health RAs
- Broader coverage of chemicals, health effects, life stages & mixtures, given the higher throughput
- Lower costs and decreased time required to obtain results
- Phased reductions in animal usage

Human Toxicology Project Consortium

DOW

DUPONT
The miracles of science™

ExxonMobil

Johnson & Johnson
PHARMACEUTICAL RESEARCH & DEVELOPMENT, L.L.C.

L'ORÉAL
Research & Innovation

P&G

Unilever

Sumitomo Corporation

THE HAMNER INSTITUTES FOR HEALTH SCIENCES

Sumitomo Corporation

THE HUMANE SOCIETY OF THE UNITED STATES

HUMAN

AXLR8

AXLR8

WHAT IS AXLR8?

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

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NEWS & EVENTS

12th Annual CEFIC LRI Workshop

'Reduction of Uncertainty Enabling Decion Making' is the theme that will be explored during this 17-18 November CEFIC workshop in Liverpool, UK, which will showcase recent results of LRI projects covering exposure and biomonitoring, omics tools, endocrine disruption, and more. Read more >

ecopa-IPAM 21st Century Toxicology Workshop

This year's ***ecopa*** annual conference is being held from 26-27 November in Milan in partnership with the Italian national platform IPAM with the theme 'toxicity testing in the 21st century and alternative methods'. Programme > Registration >

EPAA Annual Conference - 'The 2 Rs'

'Reduction and Refinement: Combining Excellence and Animal Welfare' is the theme of this year's annual conference of the European Partnership for Alternative Approaches to Animal Testing, being held 30 November in Brussels. Read more >