Frontiers in Predictive Toxicology

Thomas Hartung

frontiers in Pharmacology | Predictive Toxicology

3.8 IMPACT FACTOR



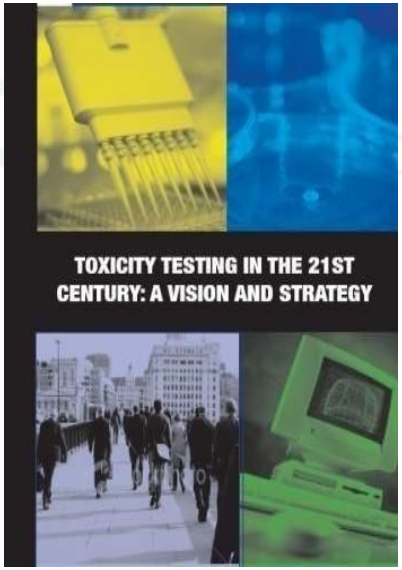

You only bother about frontiers, if you need to travel

"Away-from-here that is my destination"

Franz Kafka

1st frontier

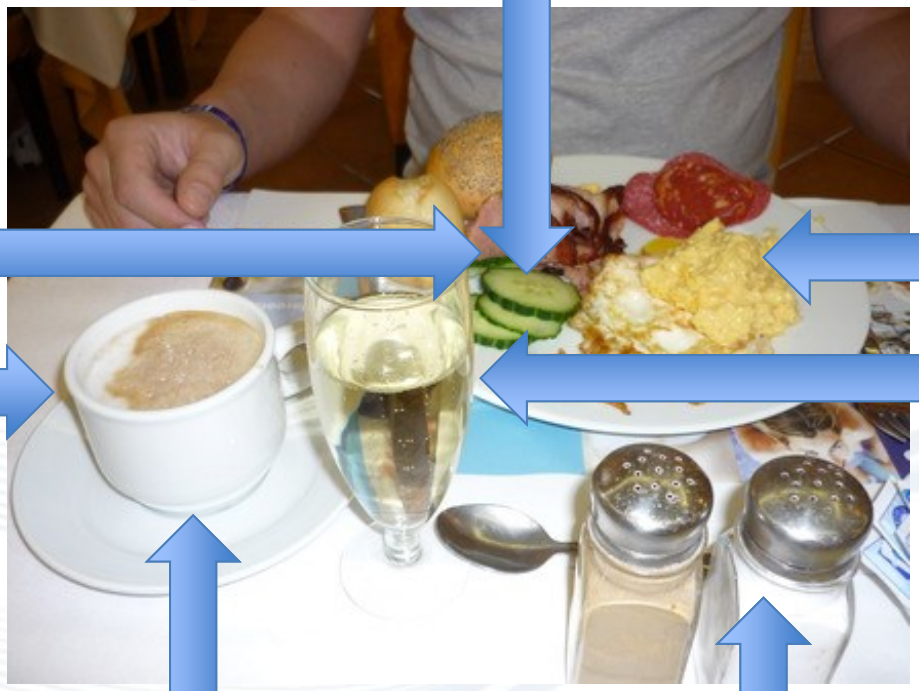
Agreement that animal tests are not good enough



Enjoy!!!

Protected against minute amounts of pesticides

Natural pesticides 10,000x more, 35 of 63 carcinogenic



Protected against TCDD in eggs

23 of 31 tested coffee ingredients carcinogenic

Same calculation for alcohol: One glass per 345 years

Genotoxic: sugar

Genotoxic: salt

We are not 70kg rats !!!

2-200 kg

20-500 g

Age 0 -100 years

Mostly 3 months, max 2 years

Different ethnics, both gender

≠

Mostly twins, one gender

Diverse food, environment

Standardized chow and cage

Disease history, Comorbidities, Multiple treatments

Healthy, Artificial diseases, Mono-treatments

Interspecies prediction of cancer

Concordance 57%

Risk Analysis, Vol. 00, No. 0, 2014

DOI: 10.1111/risa.12314

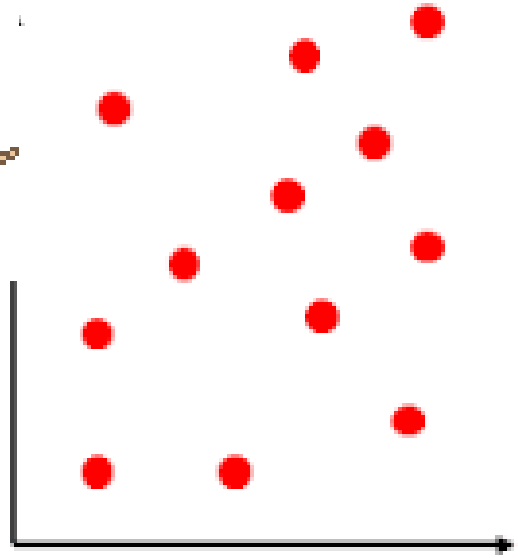
Concordanc[illegible]f Noncarcinogenic Endpoints in [illegible]odent Chemical Bioassay[illegible]

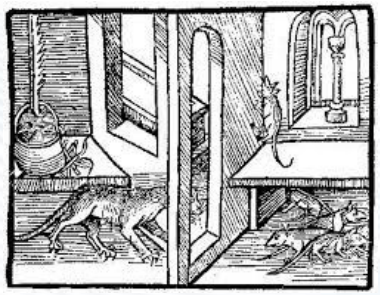
Bing Wang[1] and George Gray[2,*]

Predict[illegible] toxicologic outcomes in rodent bioassays of 37 chemicals [illegible]om the National Toxicology Program was evaluated. … Overall, there is considerable uncertainty in predicting the site of toxic lesions in different species exposed to the same chemical and from short-term to long-term tests of the same chemical.

Cancer studies in mice and rats of both gender

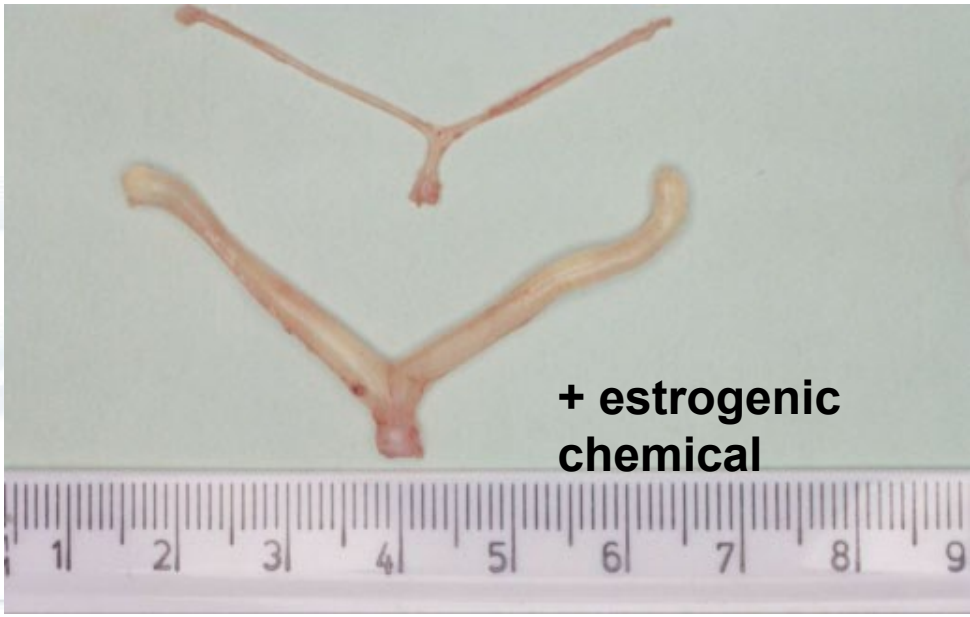
No Correlation





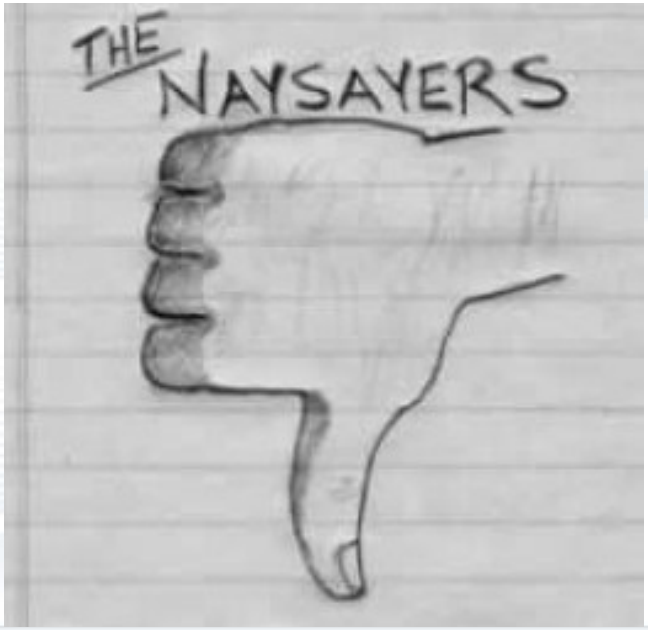
Earlier repeat-dose studies

The test solution: The uterotrophic assay on ovarectomized rats



The uterotrophic assays

OECD-"validated" test



Part of peer-review

Environ
Science &

Screening C
Computatic

Patience Browne,
and Russell S. Th

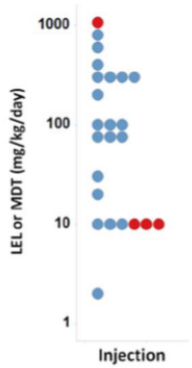


Figure 1. Variability of r
studies conducted in
methodologically similar
"guideline-like", yet have
of administration. LEL =
tested.

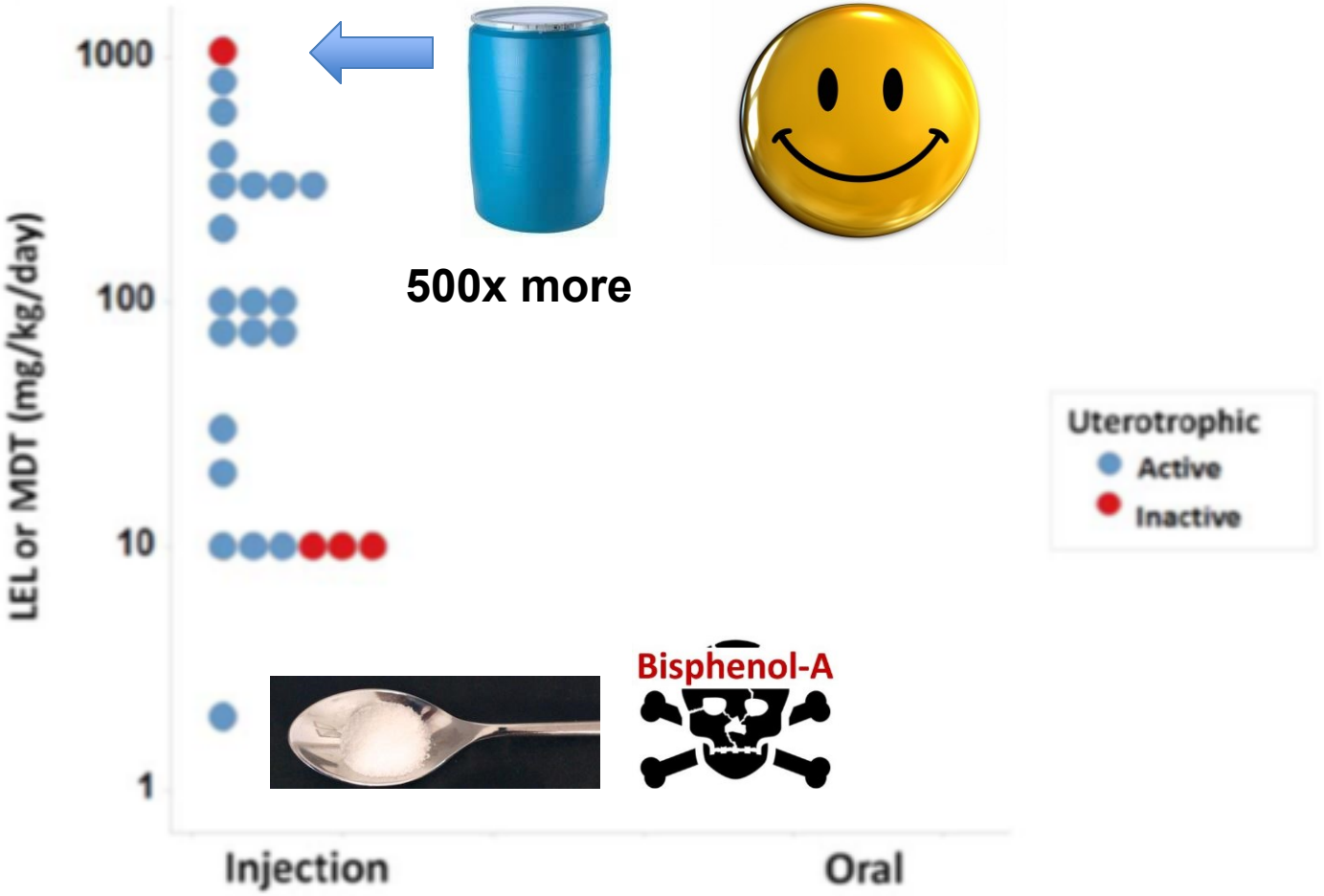


Figure 1. Variability of results for bisphenol A (BPA) in uterotrophic studies conducted in the immature rat model. All studies are methodologically similar to the EDSP Tier 1 guideline and considered "guideline-like", yet have discordant results even with the same route of administration. LEL = lowest effect level; MDT = maximum dose

"Basic research is like shooting an arrow in the air and, where it lands, painting a target."

Homer Adkins, 1984
Nature 312, 212.

Food for Thought

Look Back in Anger – What Clinical Studies Tell Us About Preclinical Work

Thomas Hartung

Johns Hopkins University, Bloomberg School of Public Health, CAAT, Baltimore, USA and University of Konstanz, CAAT-Europe, Germany

Bayer, 2011: 20-25% in-house studies reproduce publications

Amgen, 2012: 6% of cancer hallmark papers reproducible

Tab. 2: Examples of more systematic evaluations of the quality of animal studies of drug efficacy

First author	Year published	(Number of) indications	Number of studies considered (of total)	Reproducible in humans
Horn	**2001**	**stroke**	**20 (225)**	**50%**
The methodological quality of the animal studies was found to be poor. Of the included studies, 50% were in favor of nimodipine (which was not effective in human trials). In-depth analyses showed statistically significant effects in favor of treatment (10 studies) (Horn et al., 2001).				
Corpet	**200**			**55%**
"*We found that the effect of mo* Table 3 (Corpet et al., 2003) wi data or inclusion/exclusion crit in rat and mice showed a signi analysis published (Corpet et a				." Data extracted from d. No quality assurance of e. The two animal models). Updated very similar
Perel	**200**			**50% (of indications)**
"*Discordance between animal adequately.*" Poor quality of ani				*nimic clinical disease*
Bebarta	**200**			**n.a.**
"*Animal studies that do not utili than studies that employ these*				*nce between study groups*
Pound	**200**			**n.a.**
Analysis of 25 systematic revie al., 2002; Mapstone et al., 200 *potential treatments for human*				icas et al., 2002; Roberts et "*Much animal research into natic reviews.*"
Sena	**201**			**n.a.**
Analysis of 16 systematic reviews of interventions tested in animal studies of acute ischemic stroke involving 525 unique publications. Publication bias was highly prevalent (Sena et al., 2010).				
Hackam	**2006**	**diverse**	**76**	**37%**
"*Only about a third of highly cited animal research translated at the level of human randomized trials.*" (Hackam and Redelmeier, 2006)				

Systematic reviews

- **Stroke**
- **Colon cancer**
- **head injury, antifibrinolytics, stroke, neonatal resp. distress, osteoporosis**
- **Emergency med.**
- **25 other SR**
- **stroke**
- **diverse**

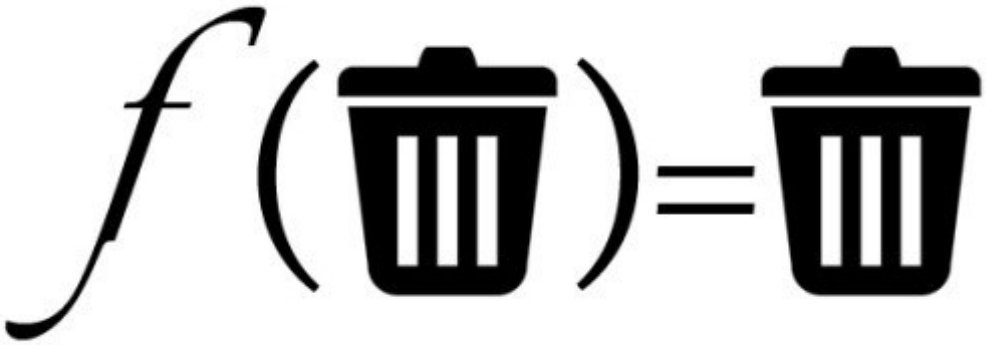
Consumer are little aware of animal testing for food

Strong discrepancies for e.g. food additives vs. pesticides

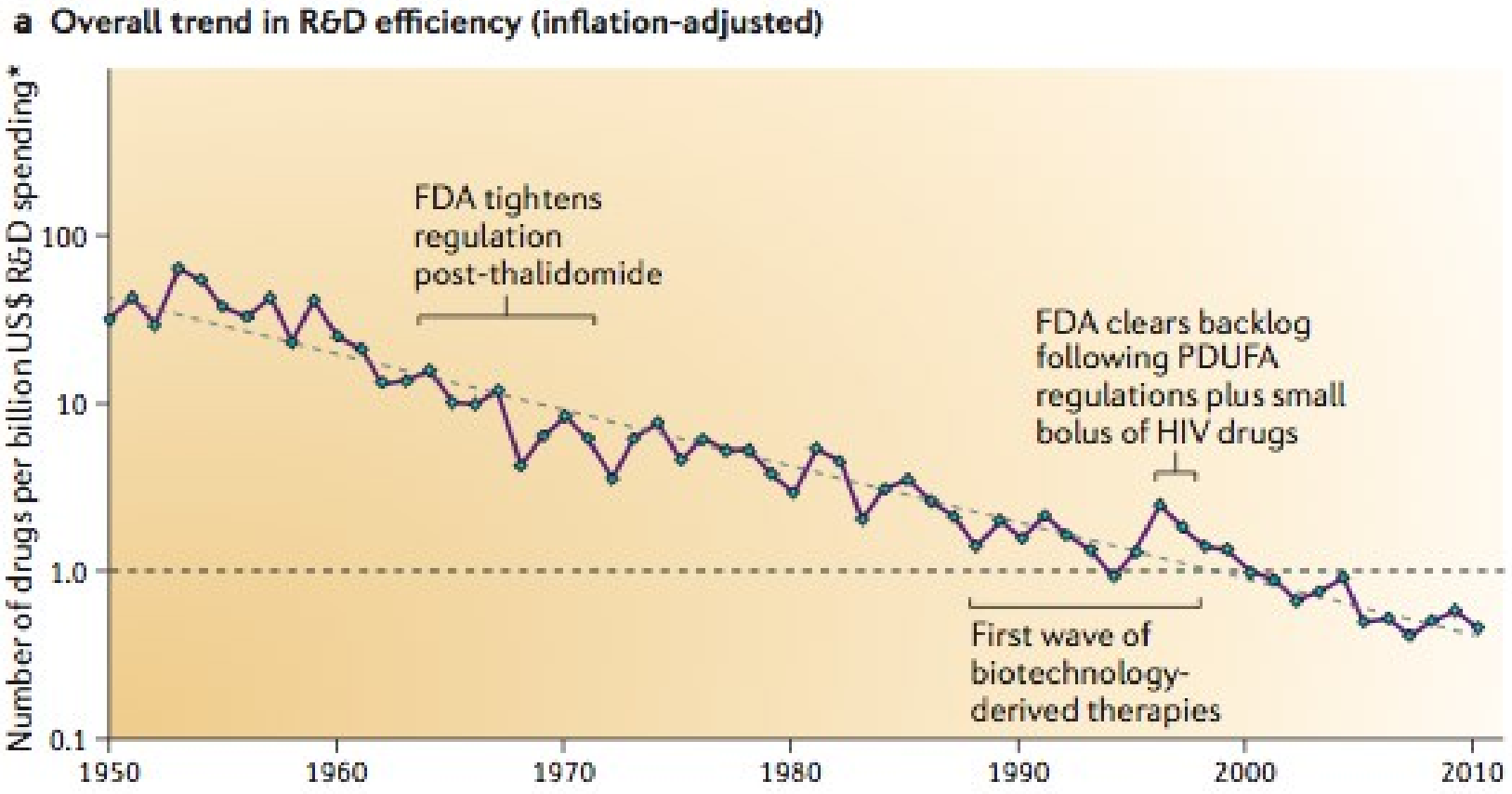
Evident data gaps (PEW report 2013), but traditional approaches not suitable

2nd frontier

Understanding that misleading animal tests make a problem



FDA-approved drugs per billion $ R&D (inflation corrected) "Eroom's Law"



Scannel et al., Nature Rev. Drug Disc. 2012

Av rage cost
$ on

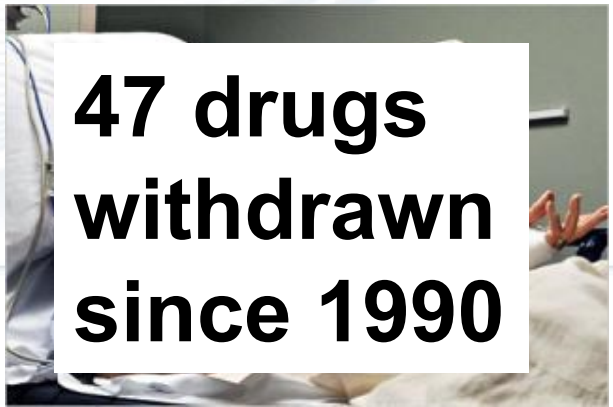
$4-11 billion
Forbes 2012

95% fail
(Arrowsmith 2012)

l:
tox not predicted
- 40% no efficacy

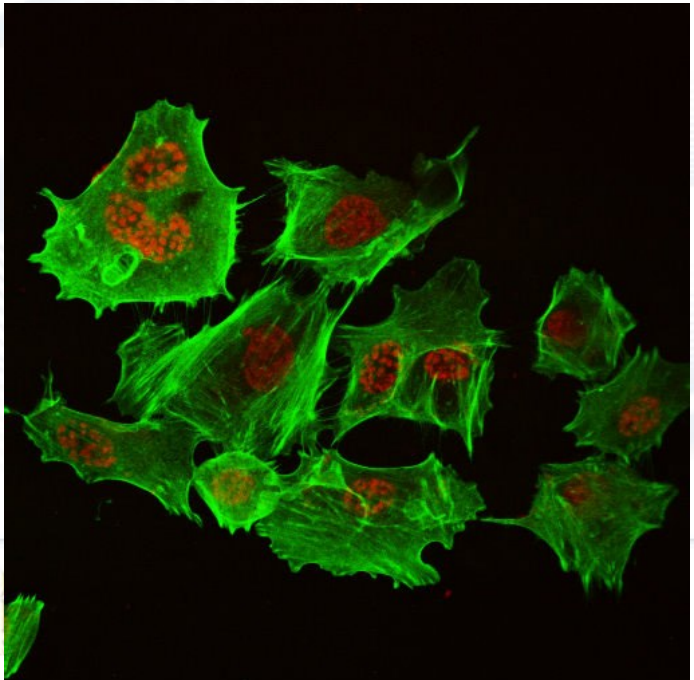
Research *Drug development* *Clinical trials*

1 in 100 patients in hospitals dies from adverse drug reactions



3rd frontier

Understanding and overcoming shortcomings of current *in vitro* tests



Limitations of in vitro mod

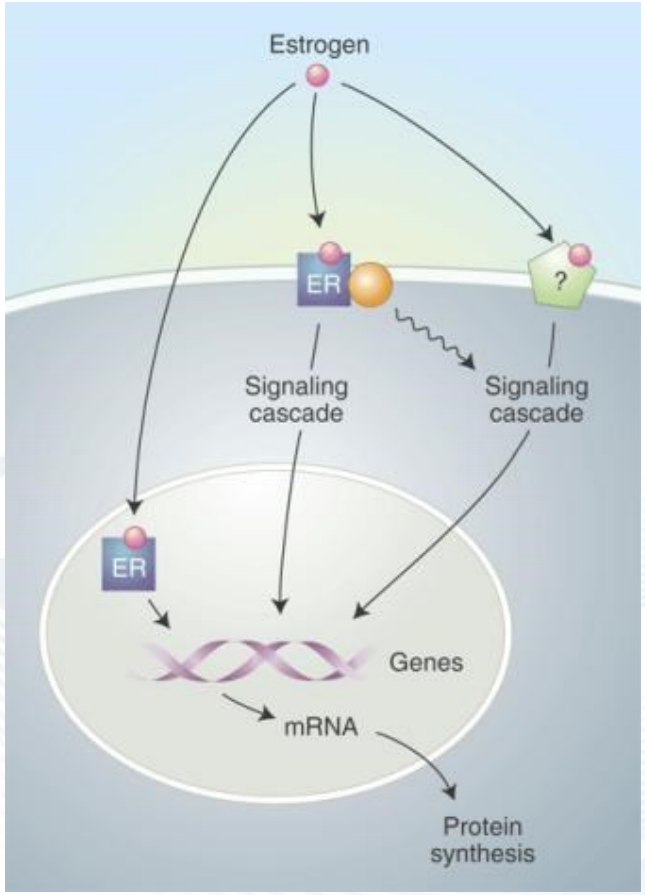
- Mycoplasma
- Dedifferenti
  growth

- etabolism and
  ise
- Unknown fate of test compounds in culture
- Tumor origin of many cells
- Cell identity

Cell models have not less limitations

Mapping the Human Toxome by Systems Toxicology

Endocrine disruption

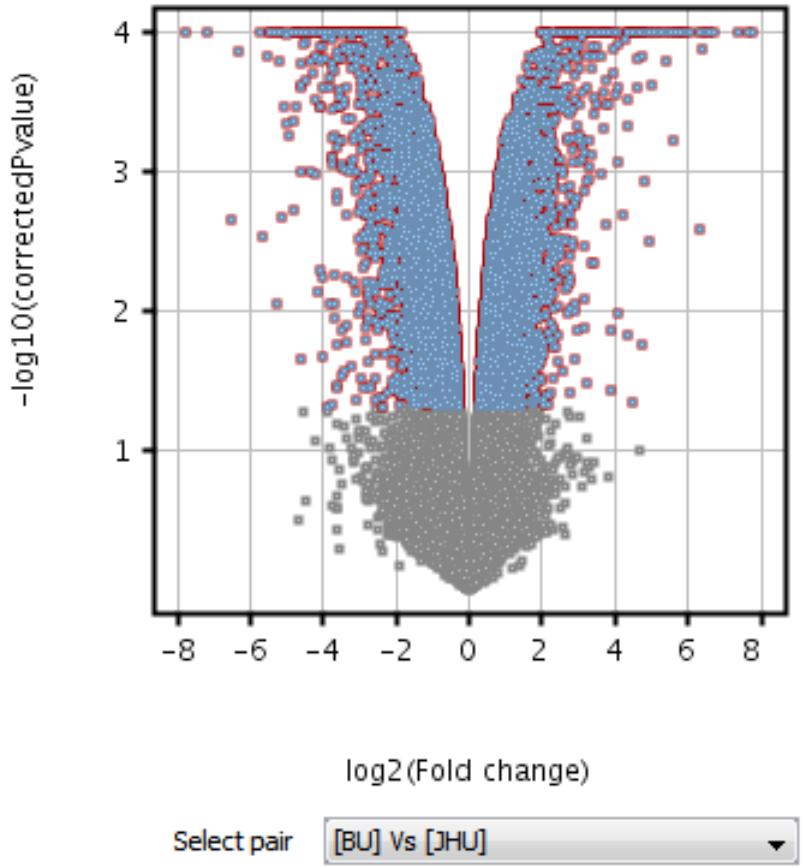


Hewitt et al., 2005. *Science*, 307:1572-1573

- **Use "omics" to map PoT for endocrine disruption**
- **Develop software tools**
- **Identify PoT**
- **Develop a process for PoT annotation, validation**
- **Establish public database on PoT.**

www.humantoxome.com

Comparison of MCF-7 in two laboratories



Same batch from ATCC

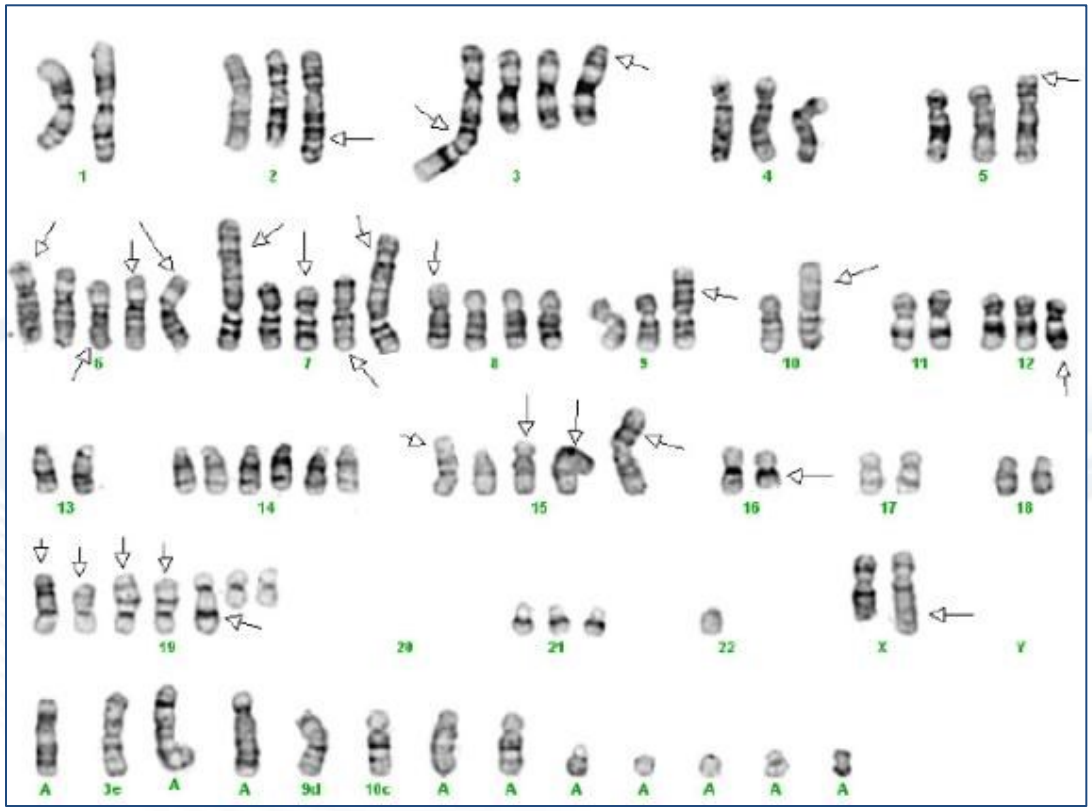
Method transfer

Transcriptomics

negative controls, 4h, gene level, *n* = 3 / group

QA of cell system is of critical importance

Good Cell Culture Practice (Coecke et al. 2005)



Karyotyping

Extent of deviations from normal genome

Classification	Kilobases	Percentage of genome
Losses	4587603	51.2%
Deletions	667374	7.5%
Amplifications	26904	0.3%
Gains	2587093	28.9%
Normal	871166	9.7%
Centromeres	217339	2.4%
Total Abberations	7868974	87.8%
All Entries	8957479	

SurePrint G3 ISCA CGH+SNP Microarray Kit, 4x180K
115234 CGH features.2440 CGH replicate probes, 59647 SNP features
reference mapping: caucasian female human reference DNA

Good Cell Culture Practice Collaboration

Coecke et al. (2005), secretariat: David Pamies

NIH National Center for Advancing Translational Sciences

FDA

DARPA

United States Environmental Protection Agency

ASCCT

UK StemCellBank

CAAT

EURL ECVAM

THE EUROPEAN UNION REFERENCE LABORATORY FOR ALTERNATIVES TO ANIMAL TESTING

NICEATM ICCVAM

Advancing Public Health and Animal Welfare

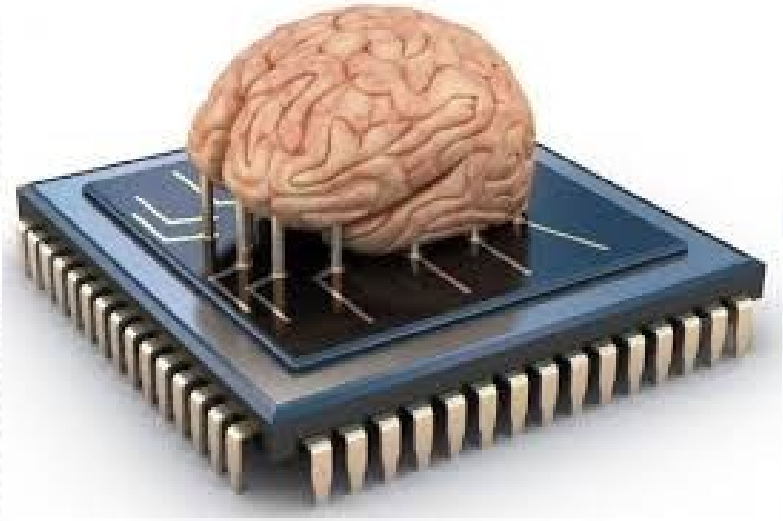
Workshops
Jun, Baltimore
Dec, Konstanz

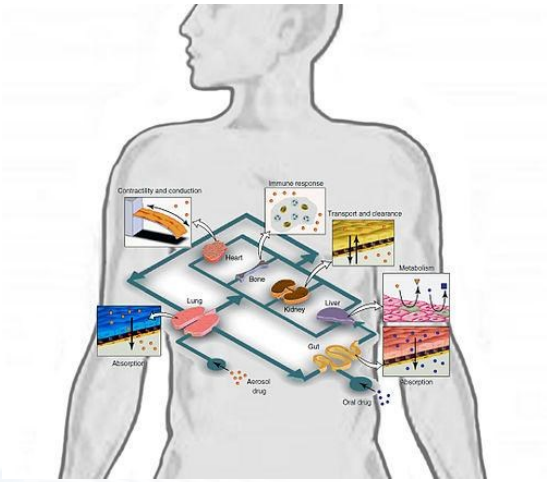
Johns Hopkins Bloomberg School of Public Health

Universität Konstanz

4th frontier

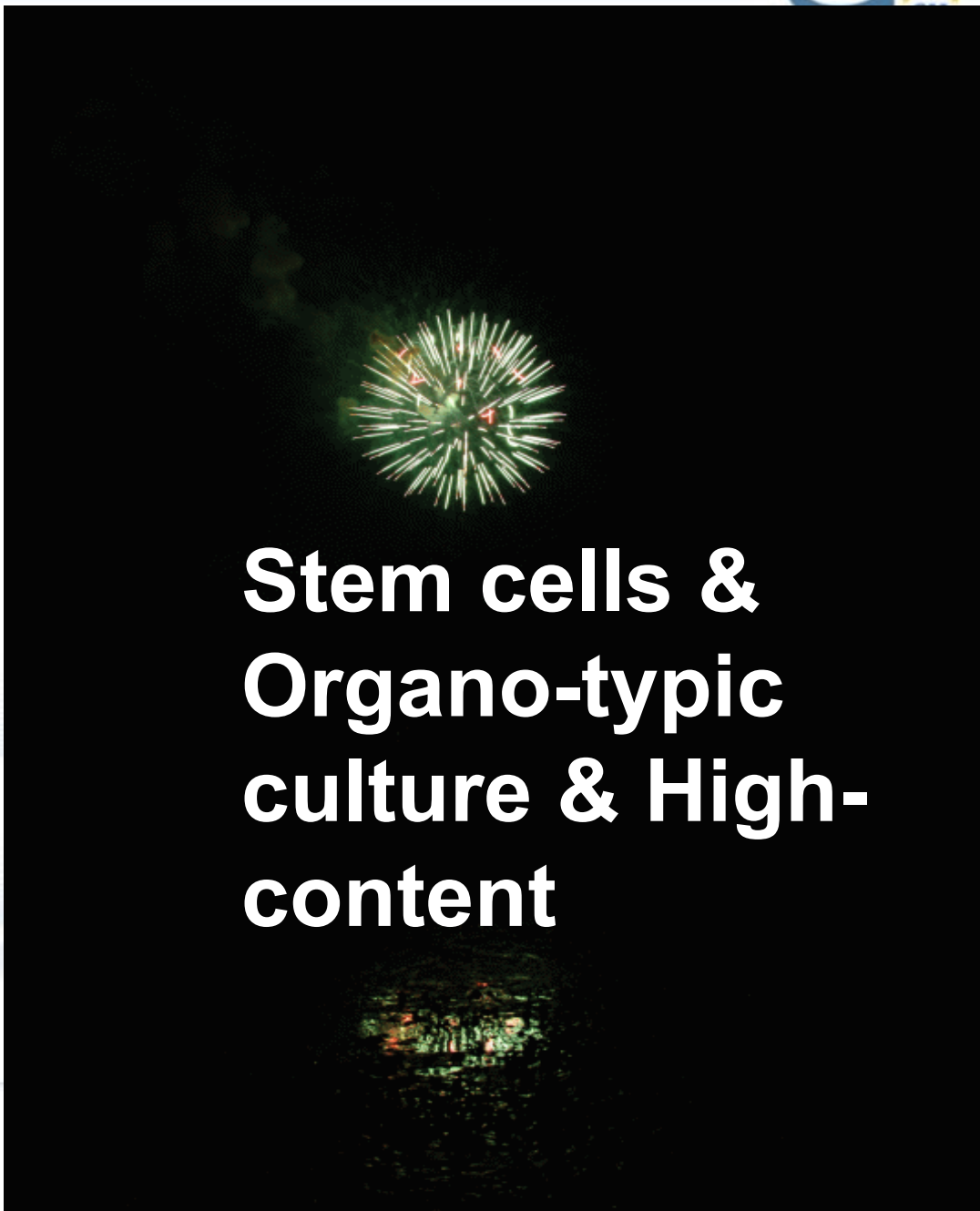
Creating organo-typic cell cultures & organs on chip



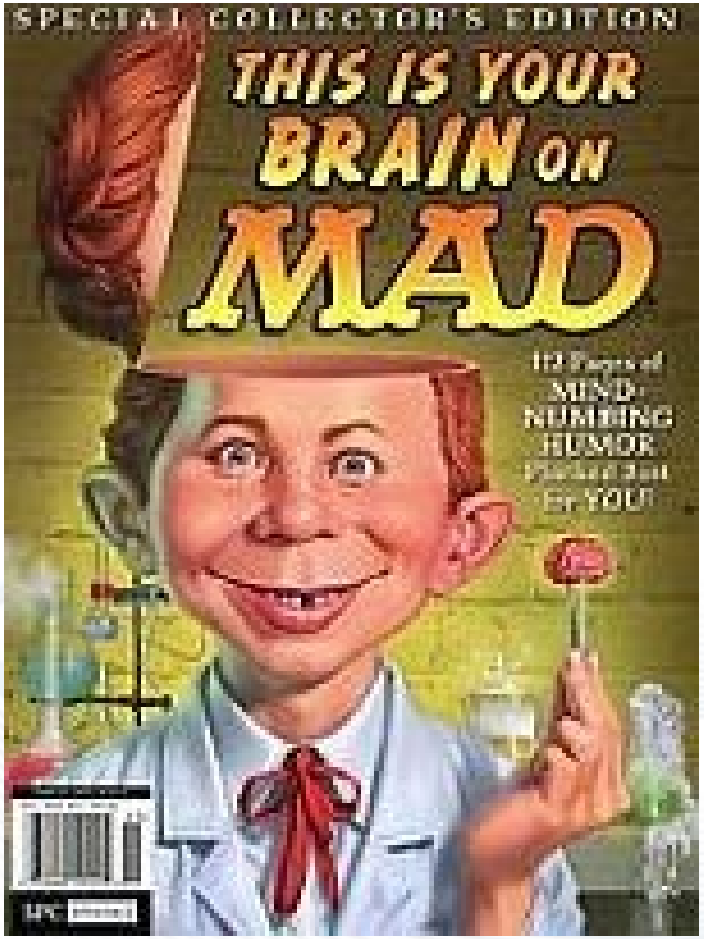


http://en.wikipedia.org/wiki/Organ-on-a-chip

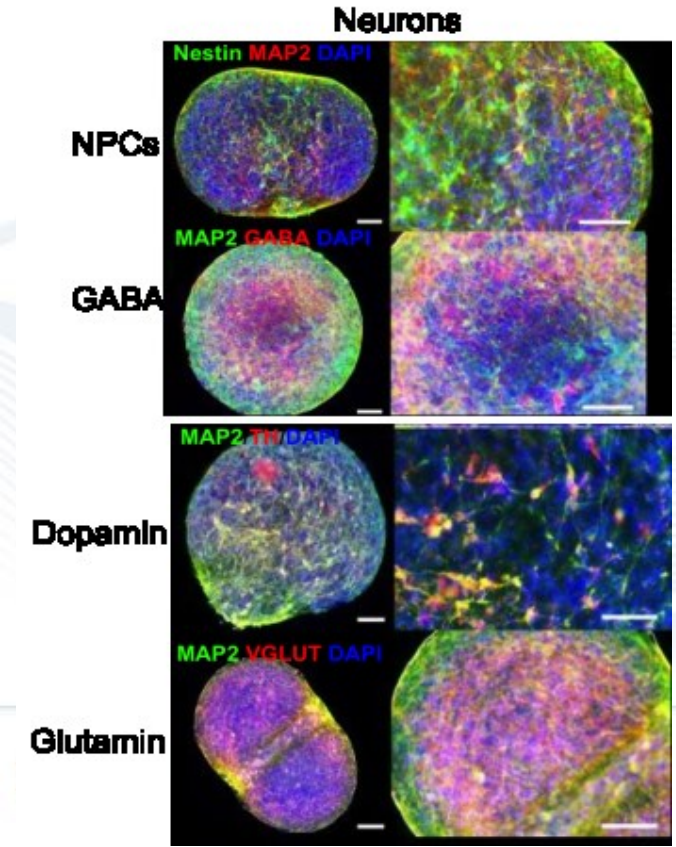
21st century toxicology starts with 21st century cell culture



Stem cells & Organo-typic culture & High-content

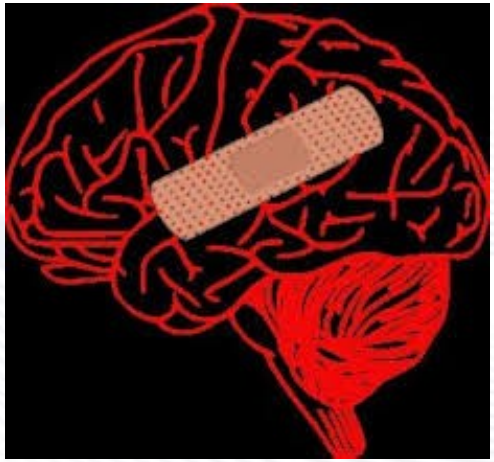


Our mini-brain project



Summary
Human "mini-brain"
developing from iPSC

- All cell types but micro-glia
- 350um diameter
- 800 per batch
- Reproducible
- Electrophysiological active
- From patient cells: gene/environment interactions



Opportunities for human mini-brain research

- **Map the neurotoxic chemical universe**
- **Characterization of medical countermeasures**
- **Neurotoxic and DNToxic side effects**
- **Brain trauma, infectious disease and neurodegenerative disease research**
- **Individual susceptibility using patient iPSC – genetic risk factors**
- **Long-term culture and co-culture with other organs**

5th frontier

Embracing other new technologies and approaches

Early Alternatives

Today

Future

Cell Culture
(one cell type, few parameters)

Organo-typic Cell Culture
(Coculture, Organ function, often Perfusion)

Human-on-chip
(Multi-Organ Models With Microfluidics)

Cell Culture + Omics or Image Analysis (high-content)

Automated Cell Culture (high-throughput Screening)

Toxicity Mechanisms
("Adverse Outcome Pathways", "Human Toxome")

Integrated Test Strategies
(combined tests)

Structure / Activity-Relationships
(Correlations)

Modeling
(Receptor binding, Virtual Organs, Kinetics)

Integrated Testing Strategies

- Many PoT = many tests
- Need for data integration
- Use of multiple information, not stand-alone replacement
- OECD: Integrated Approaches to Testing and Assessment (IATA) = ITS + kinetics + exposure + RA

Toxicology will make more use of Integrated Testing Strategies

Food for Thought ...

Integrated Testing Strategies for Safety Assessments

Thomas Hartung [1,2], *Tom Luechtefeld* [1], *Alexandra Maertens* [1], *and Andre Kleensang* [1]

[1]Johns Hopkins University, Bloomberg School of Public Health, CAAT, Baltimore, USA; [2]University of Konstanz, CAAT-Europe, Germany

t⁴ Workshop Report*

Integrated Testing Strategies (ITS) for Safety Assessment

Costanza Rovida [1], *Nathalie Alépée* [2], *Anne M. Api* [3], *David A. Basketter* [4], *Frédéric Y. Bois* [5], *Francesca Caloni* [6], *Emanuela Corsini* [7], *Mardas Daneshian* [1], *Chantra Eskes* [8], *Janine Ezendam* [9], *Horst Fuchs* [10], *Patrick Hayden* [11], *Christa Hegele-Hartung* [12], *Sebastian Hoffmann* [13], *Bruno Hubesch* [14], *Miriam N. Jacobs* [15], *Joanna Jaworska* [16], *André Kleensang* [20], *Nicole Kleinstreuer* [17], *Jon Lalko* [3], *Robert Landsiedel* [18], *Frédéric Lebreux* [19], *Thomas Luechtefeld* [20], *Monica Locatelli* [21], *Annette Mehling* [18], *Andreas Natsch* [22], *Jonathan W. Pitchford* [23], *Donald Prater* [24], *Pilar Prieto* [25], *Andreas Schepky* [26], *Gerrit Schüürmann* [27,28], *Lena Smirnova* [20], *Colleen Toole* [29], *Erwin van Vliet* [30], *Dirk Weisensee* [10] *and Thomas Hartung* [1,20]

Research Article

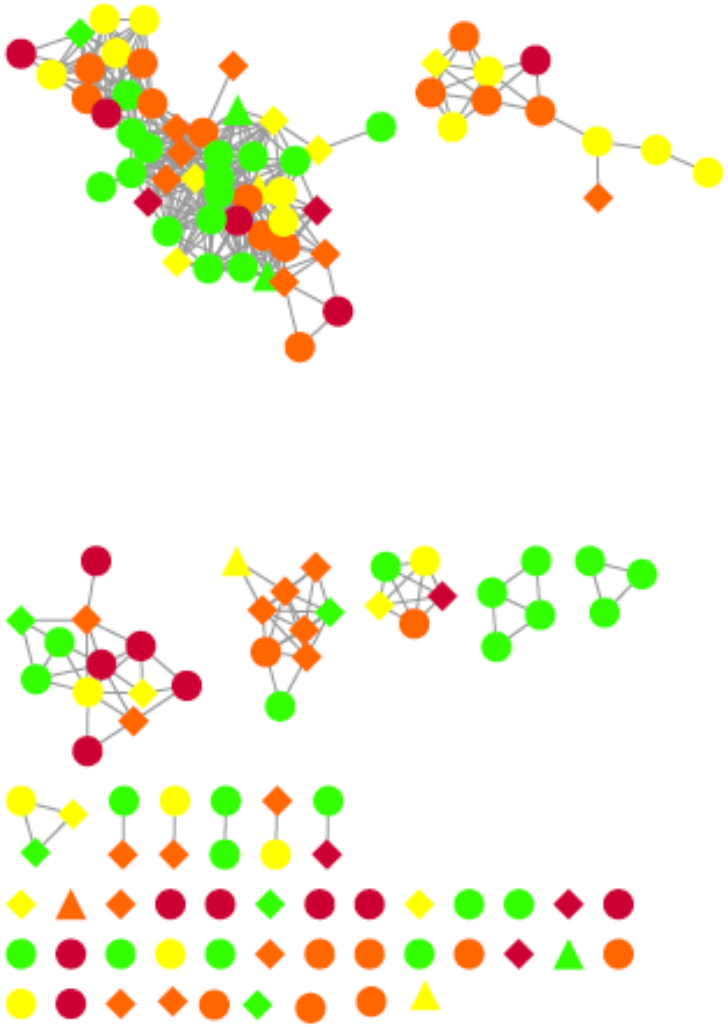
Journal of Applied Toxicology

Received: 9 February 2015, Revised: 6 April 2015, Accepted: 13 April 2015 Published online in Wiley Online Library

(wileyonlinelibrary.com) DOI 10.1002/jat.3172

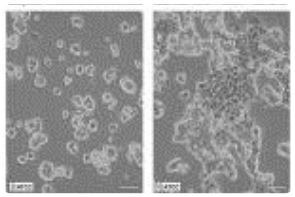
Probabilistic hazard assessment for skin sensitization potency by dose–response modeling using feature elimination instead of quantitative structure–activity relationships

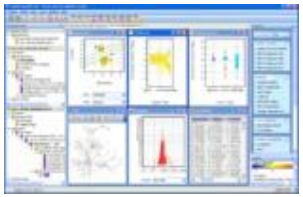
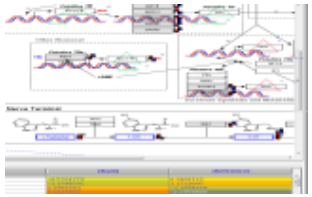
Thomas Luechtefeld[a†], Alexandra Maertens[a†], James M. McKim[b], Thomas Hartung[a,c*], Andre Kleensang[a] and Vanessa Sá-Rocha[a,d]



Chemical similarity map (Tanimoto index)

- Structure alone does not suffice
- Feature elimination as good or better than QSAR
- Supervised machine learning optimizes ITS for skin sensitization potency
- Hidden Markov Chain reduces extreme misclassification
- Accuracy 60-70%, off-by-one-class >90% standing cross-validation



In vitro model | **omics data generation** | **Software tools** | **Pathways of Toxicity** | **Validation tools** | **Human Toxome Database**

Mapping the Human Toxome by Systems Toxicology

Johns Hopkins Bloomberg School of Public Health

Georgetown University

Brown University

Agilent Technologies

The Hamner

EPA

Johns Hopkins Bloomberg School of Public Health

Universität Konstanz

Johns Hopkins University Center for Alternatives to Animal Testing

Review Article

Journal of Applied Toxicology

Received: 5 November 2012, Revised: 10 February 2013, Accepted: 11 February 2013 Published online in Wiley Online Library

(wileyonlinelibrary.com) DOI 10.1002/jat.2874

Review: Toxicometabolomics

Mounir Bouhifd[†], Thomas Hartung*[†], Helena T. Hogberg[†], Andre Kleensang[†] and Liang Zhao[†]

transatlantic think tank for toxicology

METABOLOMICS
3 WORKSHOPS
2 INFODAYS

Quality Assurance of Metabolomics

Mounir Bouhifd, Richard Beger, Thomas Flynn, Lin Guo, Georgina Harris, Helena Hogberg, Rima Kaddurah-Daouk, Hennicke Kamp, Andre Kleensang, Alexandra Maertens, Shelly Odwin-DaCosta, David Pamies, Donald Robertson, Lena Smirnova, Jinchun Sun, Liang Zhao, and Thomas Hartung

International expansion?

ChemicalWatch
GLOBAL RISK & REGULATION NEWS

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EUToxRisk21 to start this autumn

Project aims to put mechanistic-based alternative toxicity testing in a regulatory context

17 June 2015 / Europe, Risk assessment, Test/non test methods

François Busquet, Johns Hopkins University, Center for Alternatives to Animal Testing, CAAT Europe Policy Coordinator, Brussels

François Busquet: Why Europe needs the Human Toxome Project

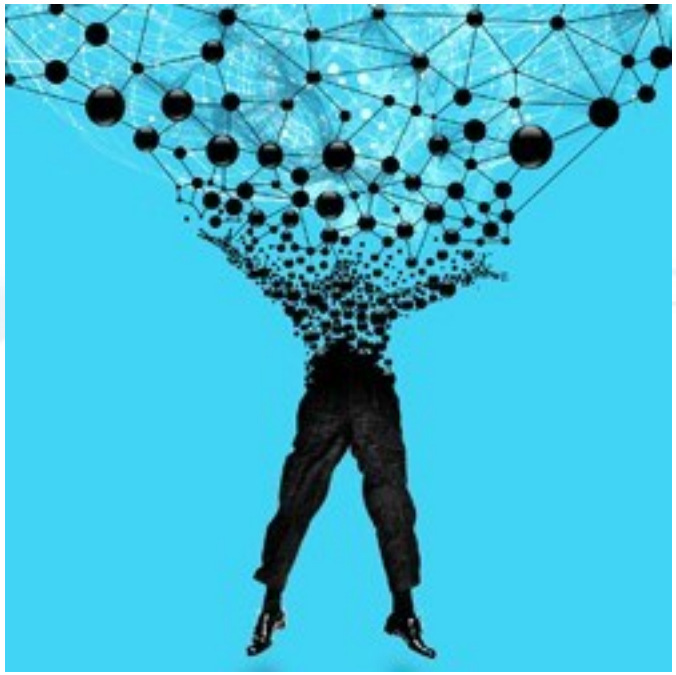
30.05.2012 - What is the Human Toxome Project? It's a wide-reaching programme aimed at helping us to reconsider how hazard/risk assessment has been performed over the last 50 years on marketed substances like chemicals, cosmetic products, pharmaceuticals, pesticides, biocides and feedstuffs.
It's necessary because most of the scientific community now accepts that animal models for testing the safety of these products have more limitations than advantages. In basic terms, the loose genetic homology to humans is no match for modern cell-culture technology, which promises to be much more apt to correctly predict toxic effects in humans. These cell cultures can

CO.DON (D)	2.80 EUR	5.26%
FLOP		
ANTISOMA (UK)	1.32 GBP	-14.84%
XENETIC BIOSCIENCES (UK)	6.00 GBP	-14.29%
PAION (D)	3.18 EUR	-7.29%

NO LIABILITY ASSUMED, DATE: 22.06.2014

6th frontier

Big data & bioinformatics



'Omics'

Image analysis

Robotised / automated testing

High throu

ToxCast™

Tox21

ig Data

Big Sense?

Big Problem!

Systems Toxicology

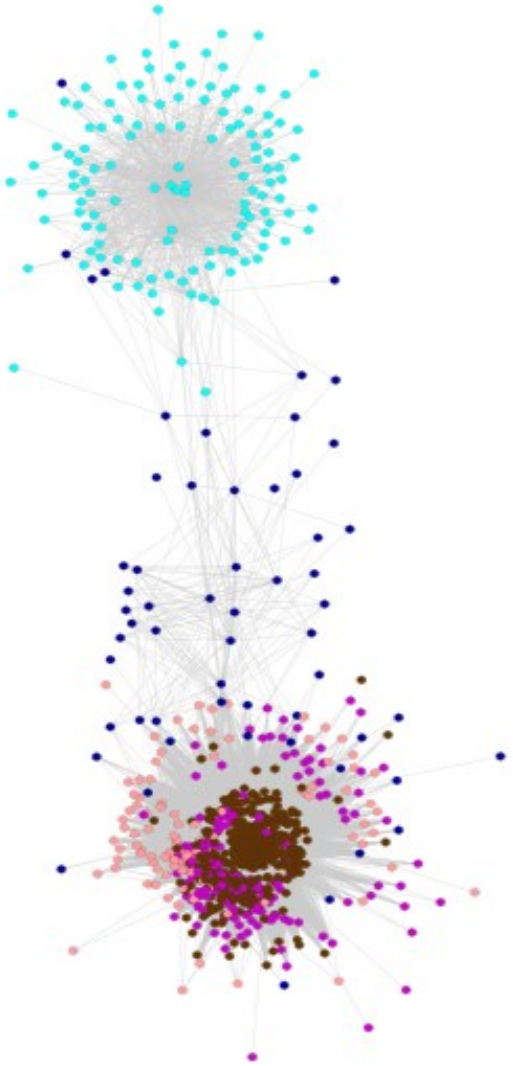


Fig. 1 Network generated by WGCNA, *colored* by module, using spring-embedded bio-layout based on edge strength

Arch Toxicol
DOI 10.1007/s00204-015-1509-6

IN VITRO SYSTEMS

MPTP's Pathway of Toxicity Indicates Central Role of Transcription Factor SP1

Alexandra Maertens[1] · Thomas Luechtefeld[1] · Andre Kleensang[1] · Thomas Hartung[1,2,3]

Arch Toxicol (2015) 89:809–812
DOI 10.1007/s00204-015-1512-y

GUEST EDITORIAL

Developing tools for defining and establishing pathways of toxicity

Melvin E. Andersen[1] · Patrick D. McMullen[1] · Daniel Krewski[2,3]

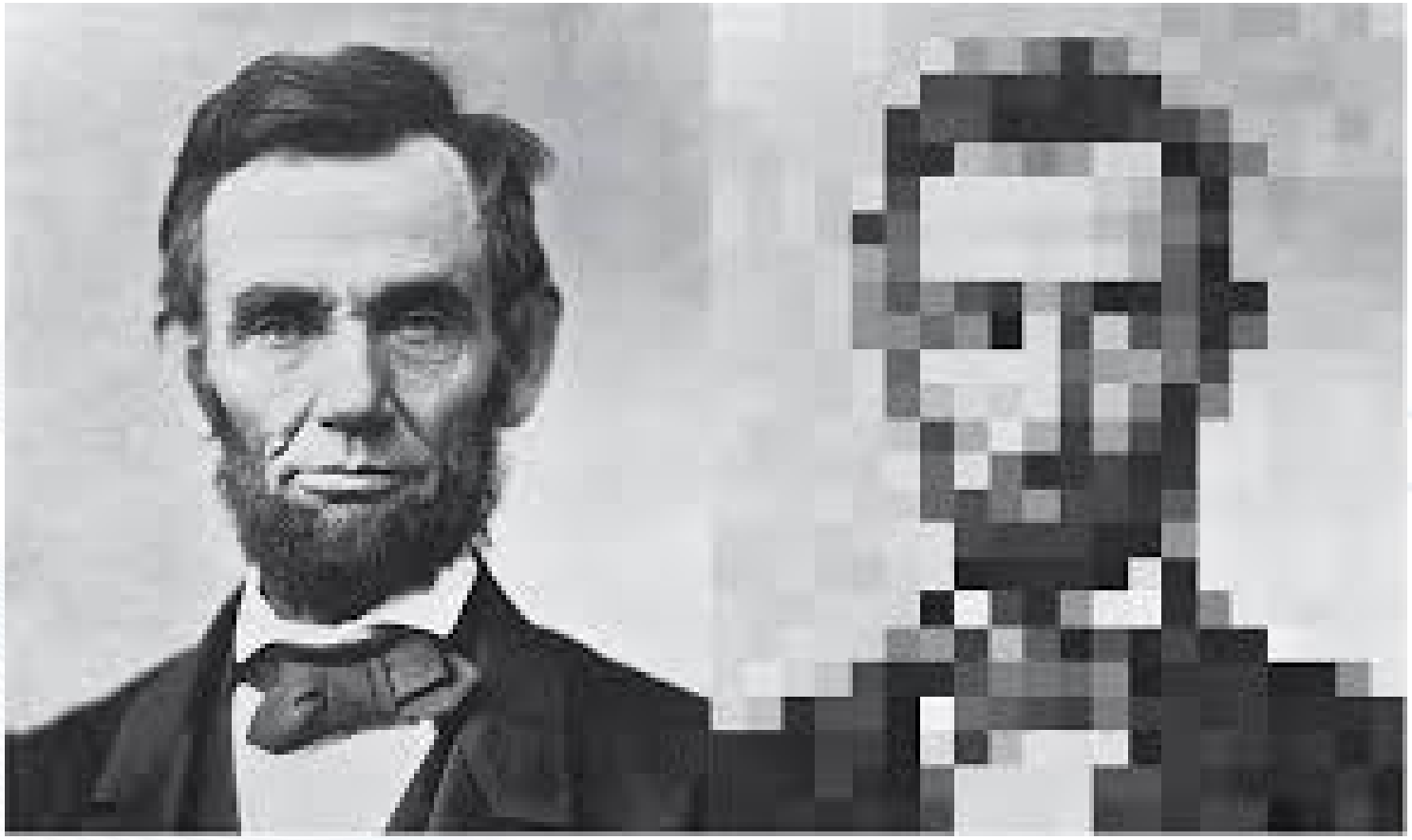
Arch Toxicol
DOI 10.1007/s00204-015-1497-6

GUEST EDITORIAL

From smoking guns to footprints: mining for critical events of toxicity pathways in transcriptome data

Jörg Rahnenführer · Marcel Leist

You don't need perfect resolution to recognize something important



7th frontier

Handling evidence differently

2006-7: Publication / 1st conference
Mar 2011: US EBTC
Oct 2011: Secretariat at CAAT
www.ebtox.com
Jan 2012: First conference hosted by EPA
Jun 2012: EU EBTC
Diverse working groups
Jul 2013: IUTOX, Seoul, Korea
Sep 2013: EuroTox, Interlaken, Switzerland
Systematic reviews increasingly embraced by EPA/IRIS, NTP and EFSA
Nov 2014: Forum Systematic Reviews
Feb 2015: FDA Training

EBT.

1st International Forum towards
Evidence-Based Toxicology (EBT)
October 15-18, 2007, Como, Italy

ebtc
Evidence-based Toxicology Collaboration

New organization in progress:

- Board of directors
- Scientific Advisory Council
- Secretariat / administration

New BoD Members

- **Jack Fowle, retired, EPA (Pres)**
- Jim Freeman, ExxonMobil
- Ian Kimber, U. of Manchester
- **Rob de Vries, SYRCLE (VP)**
- Nancy Beck, ACC
- Thomas Hartung, Hopkins
- Thomas Singer, Hoffmann-LaR.
- Andrew Rooney, NTP/OHAT

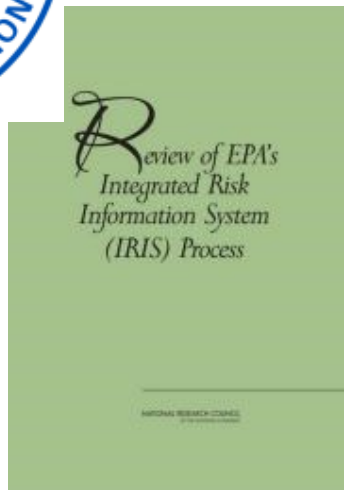
Ex officio, non-voting:
Katya Tsaouin (director), Sebastian Hoffmann & Martin Stephens

Systematic review & related approaches: Gaining acceptance in toxicology

NTP
National Toxicology Program
U.S. Department of Health and Human Services

efsa
European Food Safety Authority

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY



FDA

Feb. '15 Workshop

8th frontier

Pragmatism - Getting the rubber on the ground


GREEN TOXICOLOGY

Giving screening the green light

By working with toxicologists while they're designing new compounds, chemists can avoid problems further down the chain, as Emma Davies reports

Green Toxicology Collaboration

- Connecticut, Dec 2012
- Baltimore, Nov 2013
- Zurich, Switzerland 23 Oct 2014
- Frankfurt, Mar 2014
- SoT 2015, San Diego
- EUROTOX Sep 2016, Istanbul

- TRAINING !!!

Read-across Collaboration

ALTEX 2014, 31:387-396

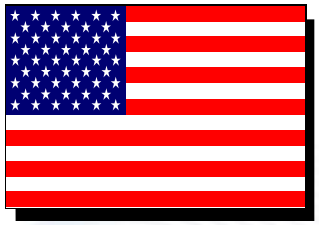
Food for Thought ...
Read-Across Approaches – Misconceptions, Promises and Challenges Ahead

Grace Patlewicz[1], Nicholas Ball[2], Richard A. Becker[3], Ewan D. Booth[4], Mark T. D. Cronin[5], Dinant Kroese[6], David Steup[7], Ben van Ravenzwaay[8] and Thomas Hartung[9]*

International Steering Group & Whitepaper
Workshop in Baltimore Oct 8-9, 2015
"Good Read-across Practice"
Stakeholder Fora Brussels & Washington early 2016

9th frontier

Managing the trans-Atlantic divide



Top-down development of new toxicological tools

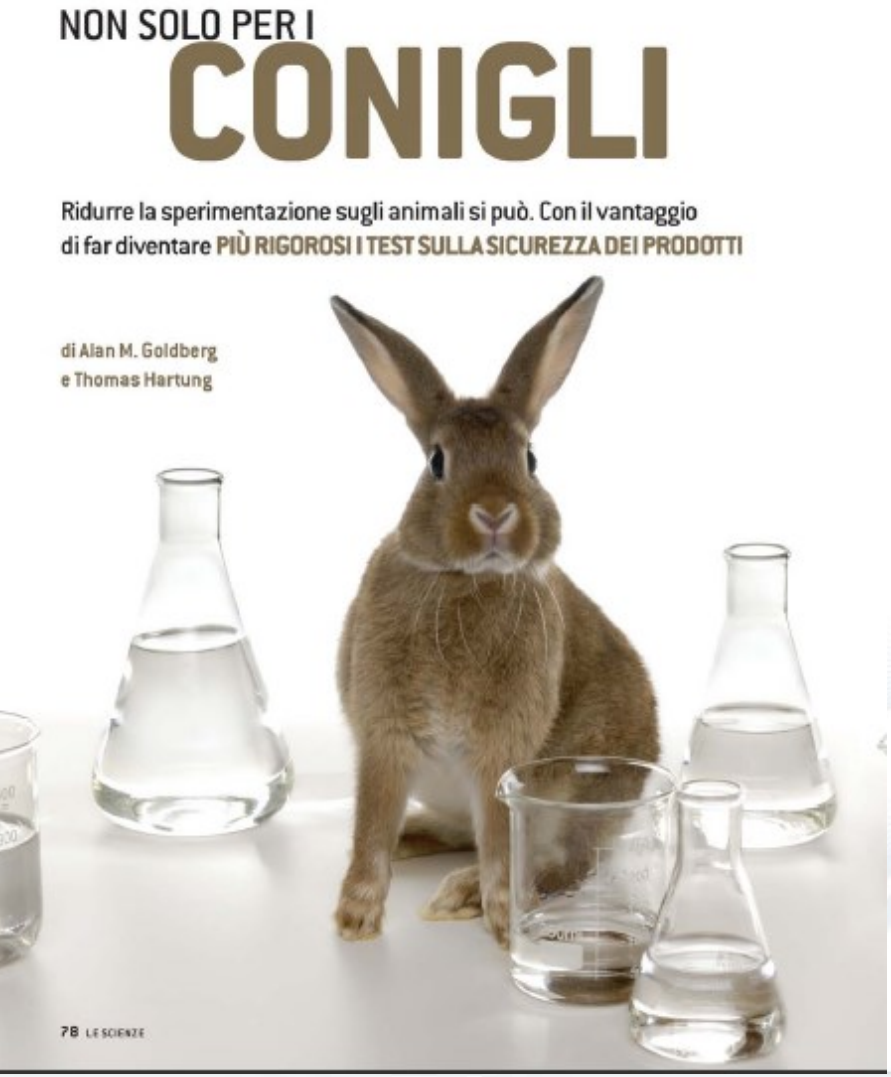
Tox-21c

3Rs

Johns Hopkins University Center for Alternatives to Animal Testing

Bottom-up support to alternative methods and legislative pressure

Scientific American 2005



- Better science
- Less animals
- Human relevance
- Faster and cheaper results
- Refinement

- Information, Grants
- Think tank
- New tools, quality control
- EU branch, policy program
- Stakeholder consensus

Funding from industry, philanthropy and research funding agencies

ALLERGAN

RIFM

IFRA INTERNATIONAL FRAGRANCE ASSOCIATION

GMA

BDF Beiersdorf

Johnson & Johnson

Agilent Technologies

NOVARTIS

Abbott A Promise for Life

Pfizer

Working together f

DOW

ExxonMobil

Boehringer Ingelheim

L'ORÉAL

Roche

P&G

Unilever

BASF The Chemical Company

JOHNS HOPKINS BLOOMBERG SCHOOL of PUBLIC HEALTH

PEPSICO

The Bernice Barbour Foundation

DOERENKAMP-ZBINDEN Foundation

THE ESTHER A. & JOSEPH KLINGENSTEIN FUND, INC.

…and individuals

EUROPEAN COMMISSION

FDA

NATIONAL INSTITUTES OF HEALTH

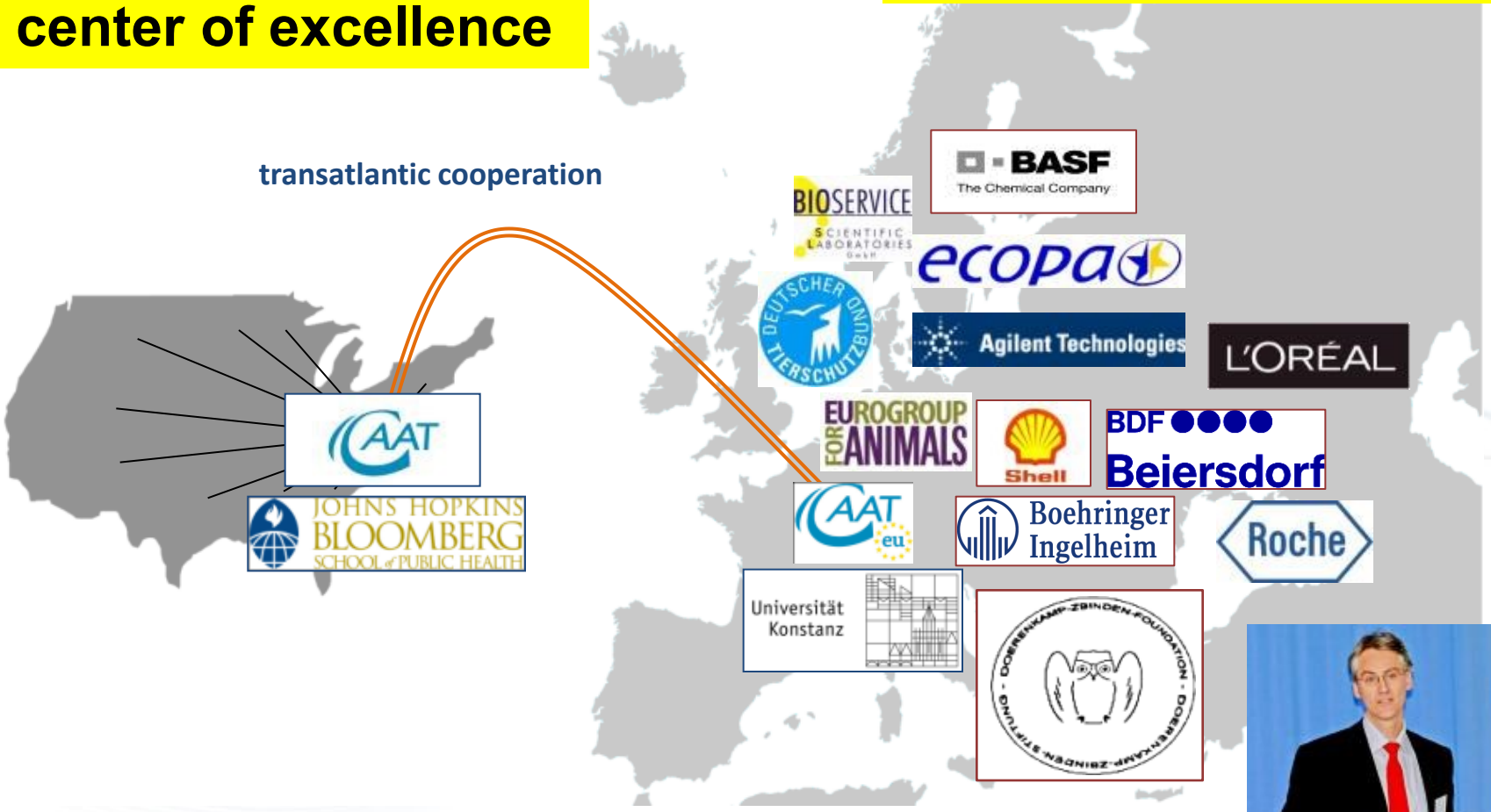
DEFENSE THREAT REDUCTION AGENCY UNITED STATES OF AMERICA

CAAT-Europe 2010

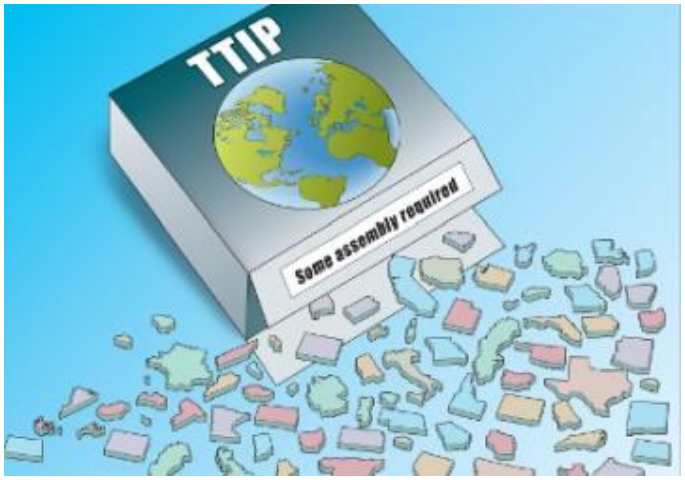
US Policy program 2006
EU center of excellence

EU Policy program 2012

transatlantic cooperation



Daneshian et al. (2010) Altex, 27, 63 – 69.



Challenge and opportunity of TTIP for harmonization of regulation

10th frontier

In a global market, no new method will be used until the last important region accepts it.

Toward humane science

CAAT

Russell and Burch

In vitro testing

ECVAM

Validation

Tox-21c

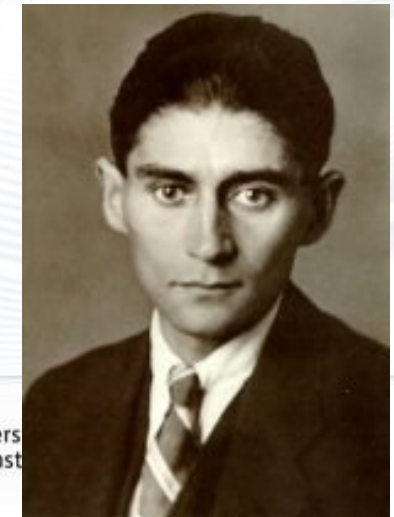
Yes, we can! Let's do it!

"There is a destination

but no way:

what we call 'The Way',

is our hesitation."



Franz Kafka