Johns Hopkins University Center for Alternatives to Animal Testing

A 3D model of human brain development for studying gene/environment interactions

Thomas Hartung

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Bloomberg School of Public Health, Johns Hopkins University, Baltimore, US

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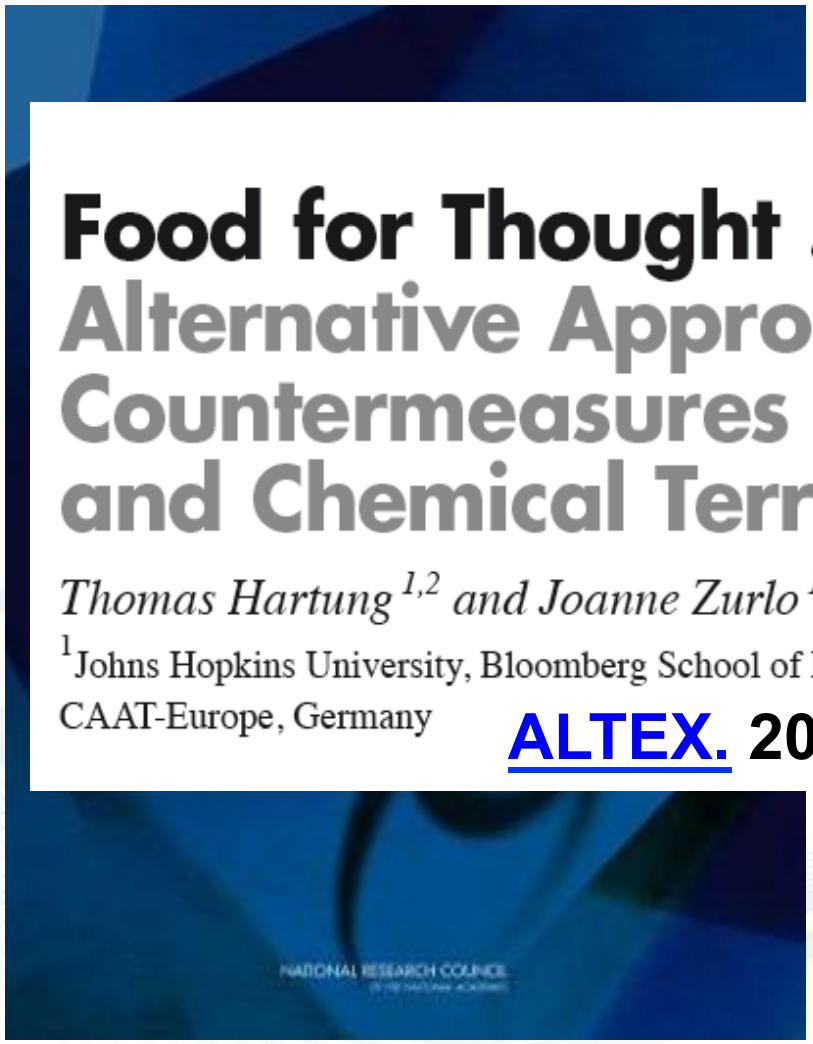
Chemicals

Pathogens

Terrorism & Warfare

Drugs & Countermeasures

… but no patients to test on.



NAS committee

warranted at this time … utilizing alternative methods to animal models"

Average cost
$1,4 billion

92% fail:
- **20% tox not predicted**
- **40% no efficacy**

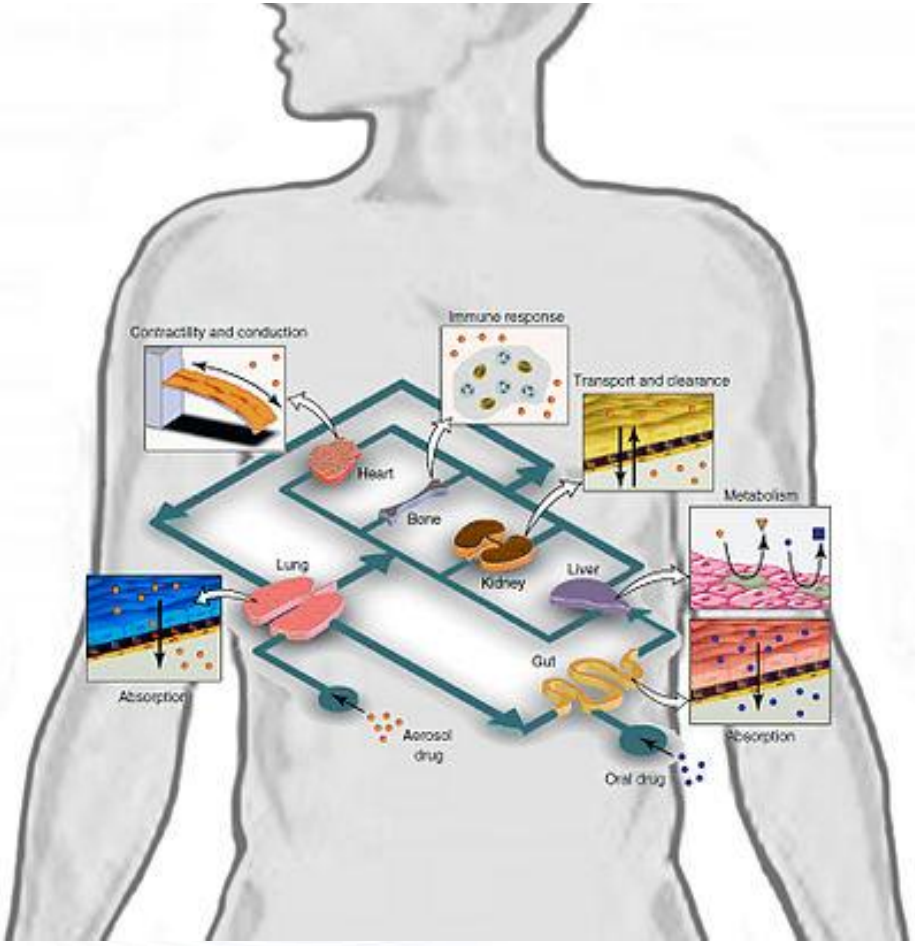
Research ***Drug development*** ***Clinical trials***

1 in 100 patients in hospitals dies from adverse drug reactions

Limitations of (animal-based) drug development

- **Humans are not 70 kg-rats…**
- **Young animals, artificial diseases, unrealistic treatments, lack of covariables (comorbidity, other treatments)**
- **Few evaluations, e.g. stroke, sepsis, multiple sclerosis, show disappointing results**
- **Lack of reproducibility by industry of academic preclinical studies (11-25%)**

Human on Chip Approach



Could overcome many of these shortcomings, especially using stem cells

C. Zhang et al. (2009), "Towards a human-on-chip: Culturing multiple cell types on a chip with compartmentalized microenvironments"

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

CAAT The Johns Hopkins Center for Alternatives to Animal Testing

CAAT Information Day
Tuesday, May 22, 2012
10:00 am – 4:30 pm
Sheldon Hall (W1214)
Johns Hopkins Bloomberg
School of Public Health
615 North Wolfe Street
Baltimore, MD

New Approaches to Assessing Countermeasures to Bioterrorism Agents

Speakers include:

George Korch (JHBSPH and US DHHS)
William C. (Clint) Florence (DTRA)
Donald Drake (Sanofi-Pasteur)
Marti Jett (US Army)
Anthony Bahinski (Wyss Institute, Harvard)
Sonia Grego (RTI International)
Lisa Hensley (US FDA)
Thomas Hartung (CAAT)

Registration fee (including lunch): $100 (free for the JHU community)
For registration and information, contact Marilyn Principe at mprincip@jhsph.edu

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Opportunities from countermeasures to bioterrorism

- **Funding program ($200 million) from NIH/FDA/ DARPA/DTRA**
- **Need for predictivity, QA, validation**
- **Joint workshop 10 May 2013 FDA / NIH / DARPA / CAAT**

NCATS Catalyzing Innovation

DEFENSE THREAT REDUCTION AGENCY · UNITED STATES OF AMERICA

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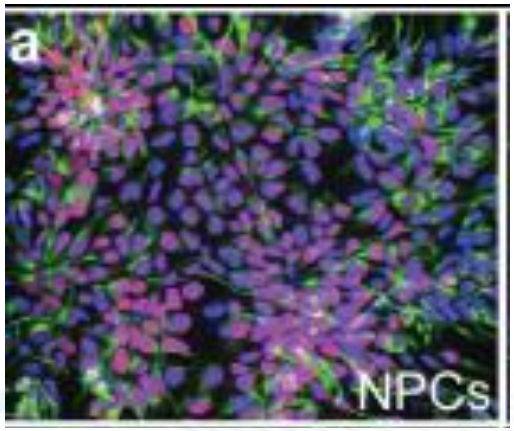
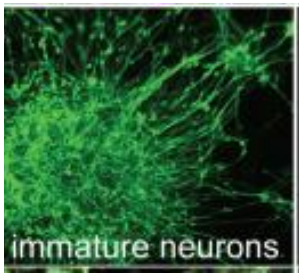
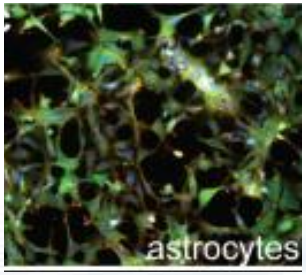
InfoDay 22 May

Preparation of 3D aggregating brain cell cultures from human iPSCs

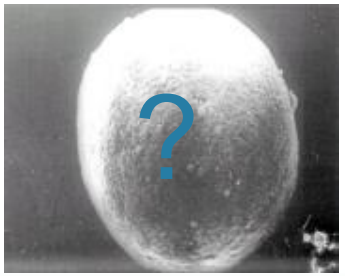






School of Medicine

School of Public Health



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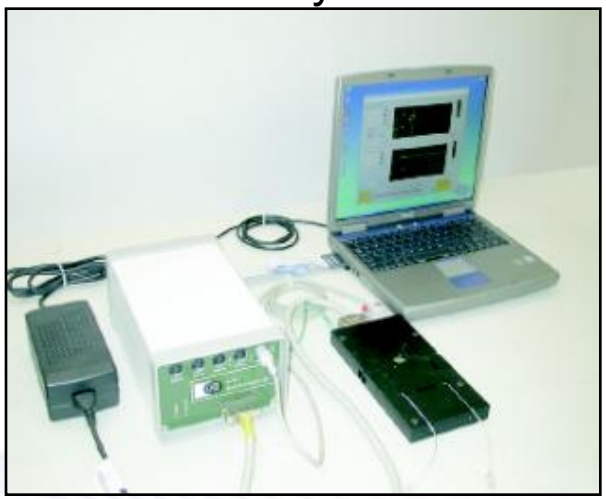
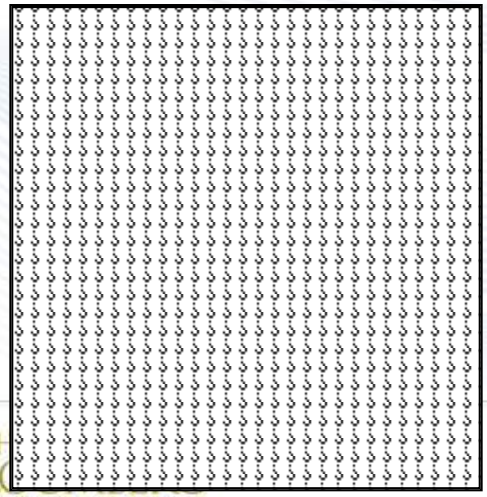
Multi-electrode array (MEA) recordings in aggregates

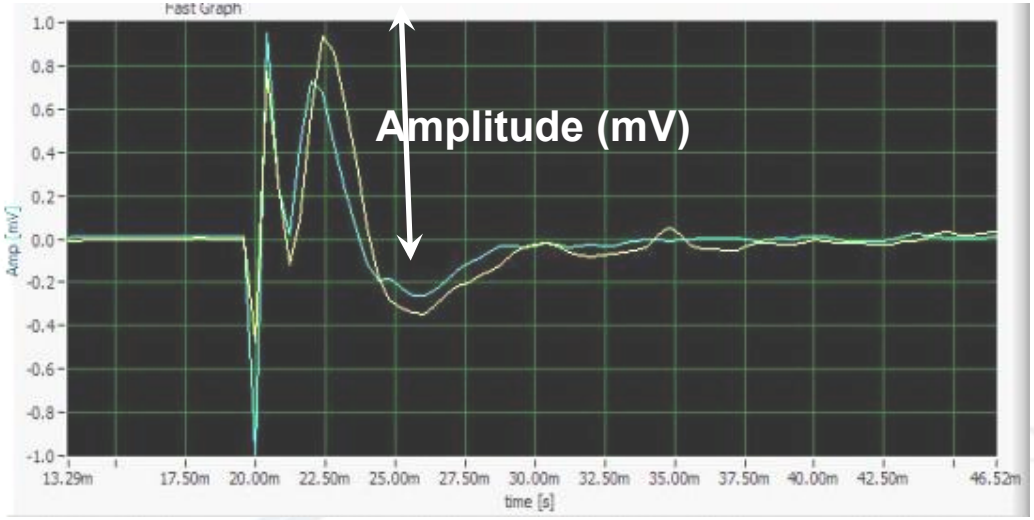
MEA system



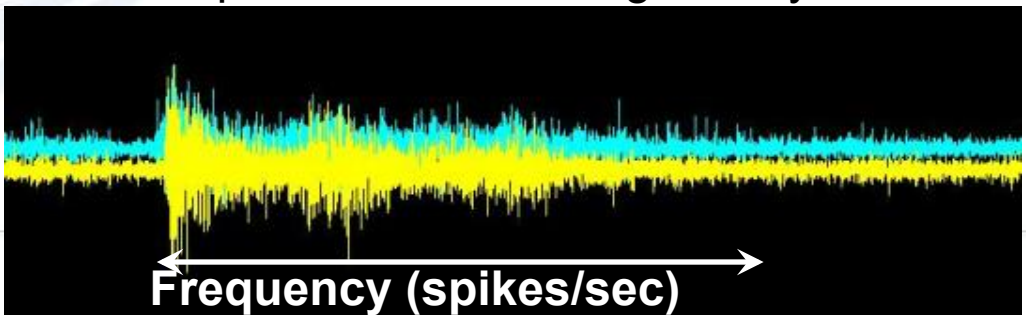
Aggregate on MEA



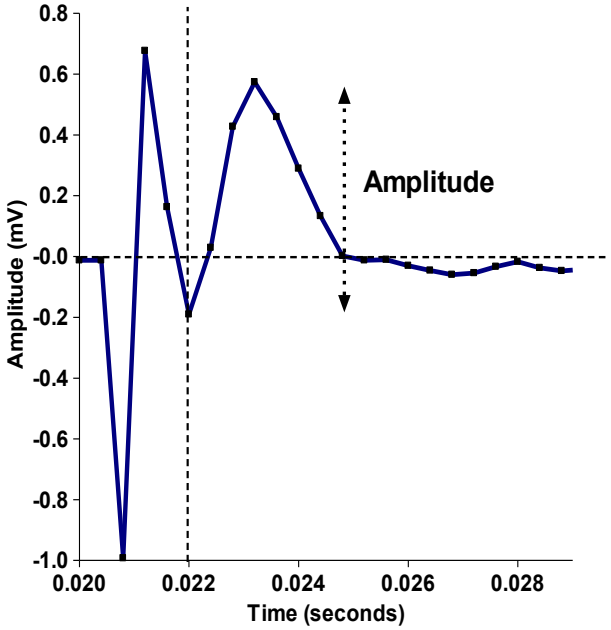
Evoked field potentials



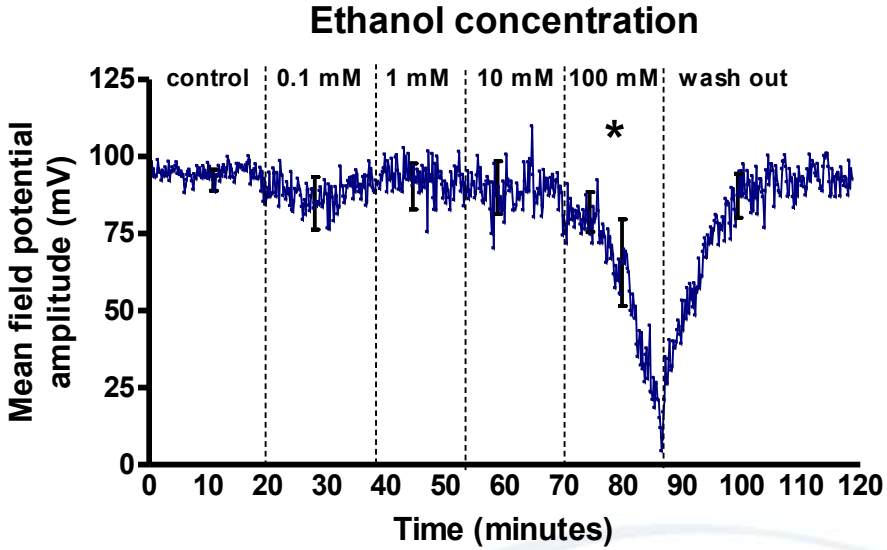
Spontaneous bursting activity

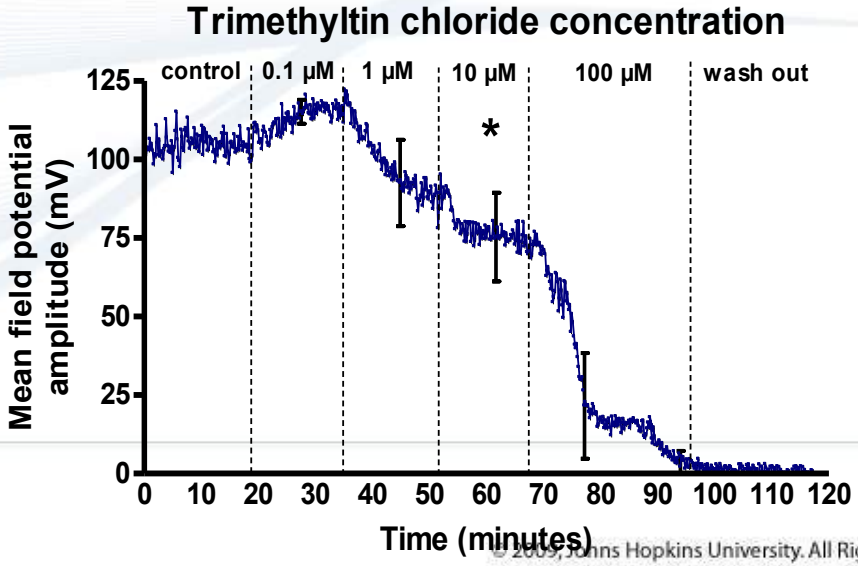


Prediction of neurotoxicity by MEA recordings

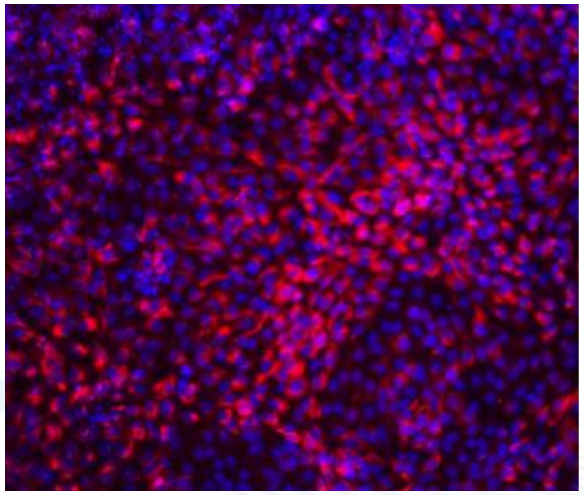


van Vliet et al., Neurotoxicology, 2007.

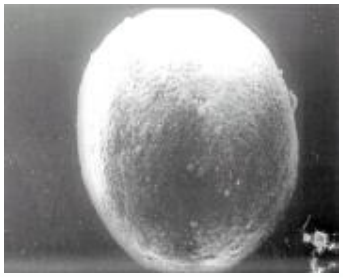




Preparation of 3D aggregating brain cell cultures from human iPSCs

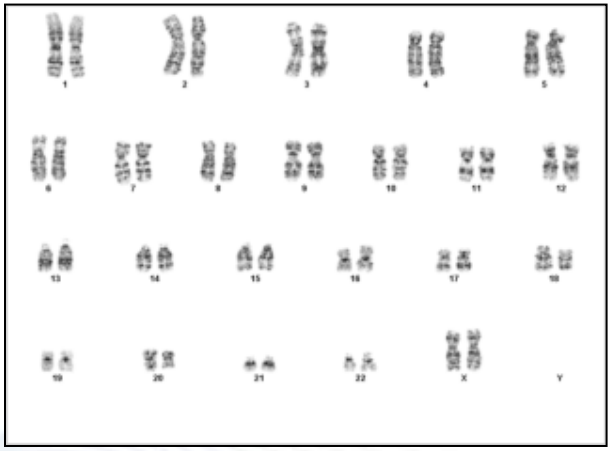


NPC



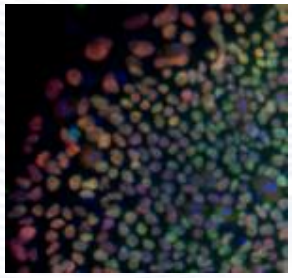
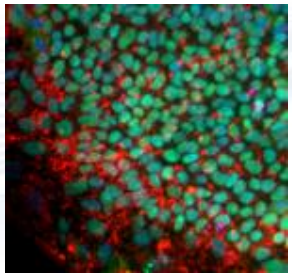
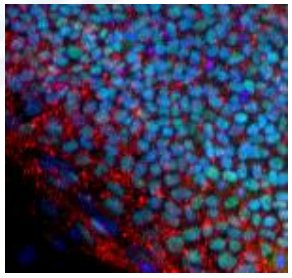
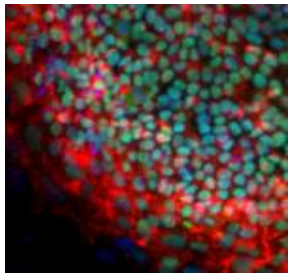
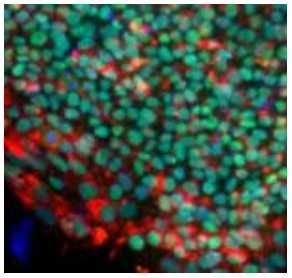
Characterization of human iPSCs

KARYOTYPING

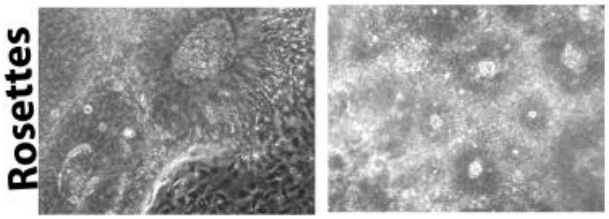


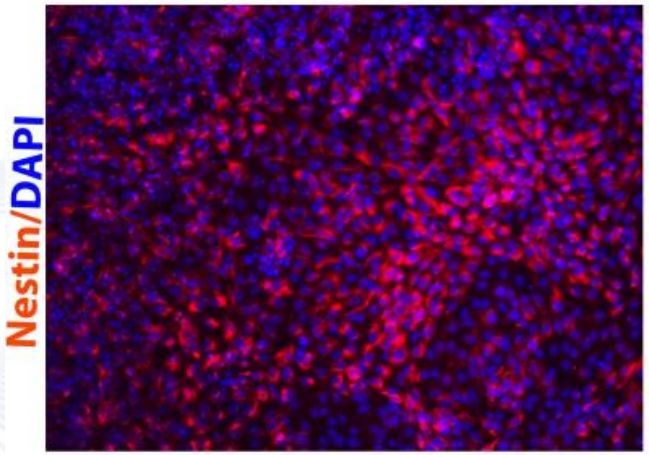
School of Medicine

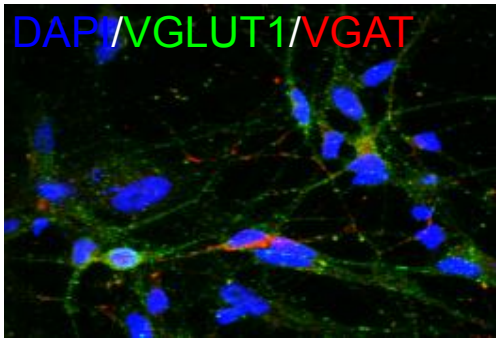
PLURIPOTENT MARKERS

Nanog/Oct4/ DAPI	Sox2/SSEA3/ DAPI	Sox2/SSEA4/ DAPI	Sox2/ Tra-1-60/DAPI	Sox2/ Tra-1-81/DAPI
				

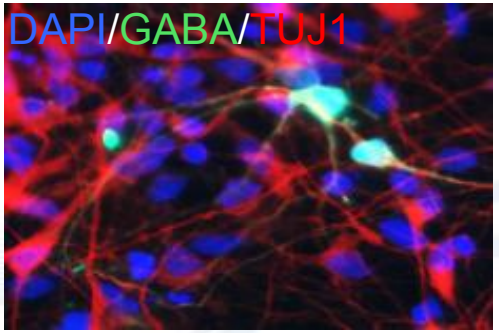
Neuronal Differentiation



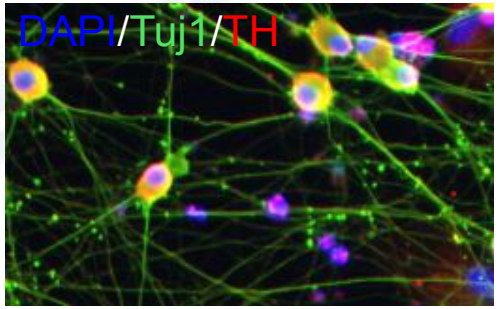




Glutamatergic

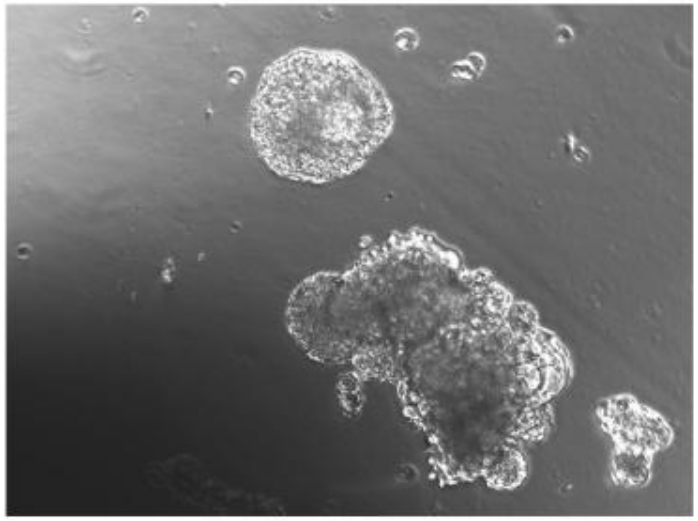


GABAergic

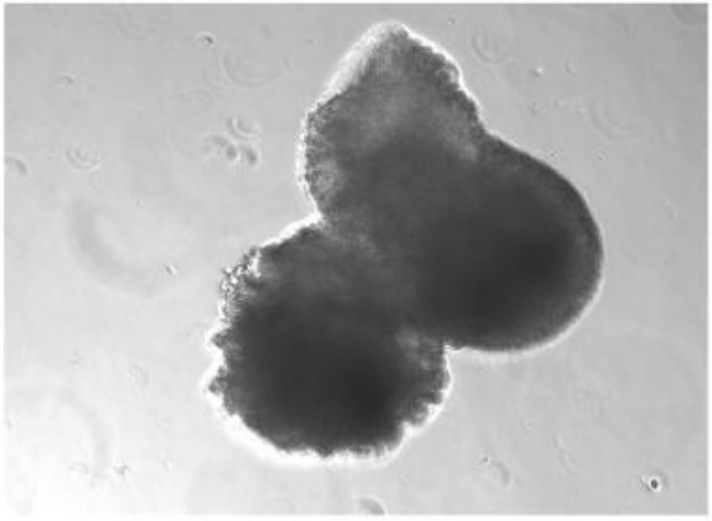


Dopaminergic

3D aggregating brain cell cultures from human iPSCs

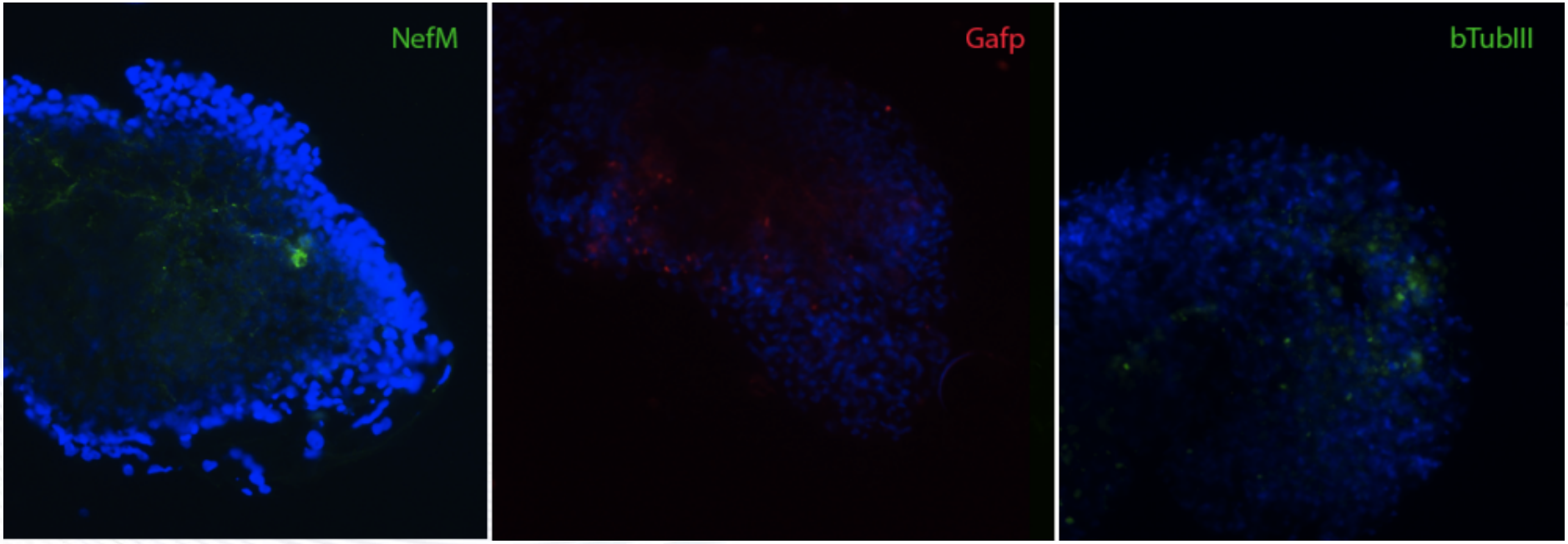


4 DIV

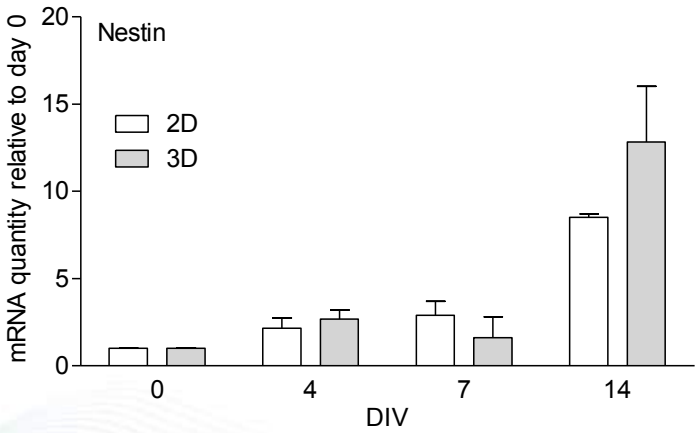


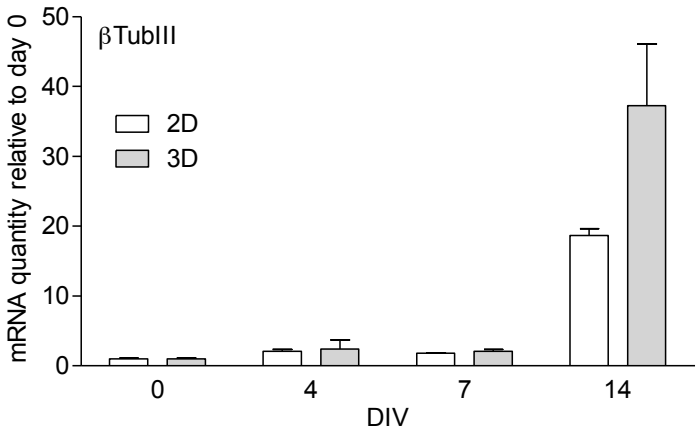
7 DIV

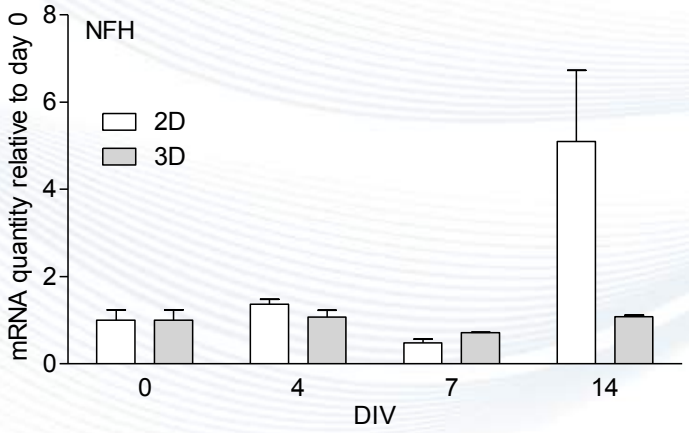
3D aggregating brain cell cultures from human iPSCs at 7 DIV

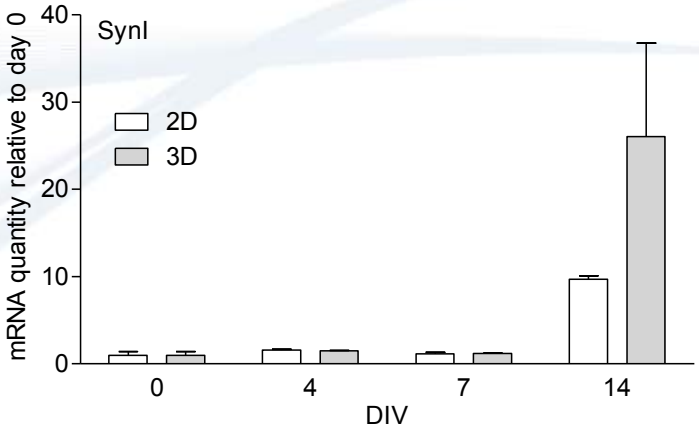


mRNA expression in 2D and 3D neuronal cultures



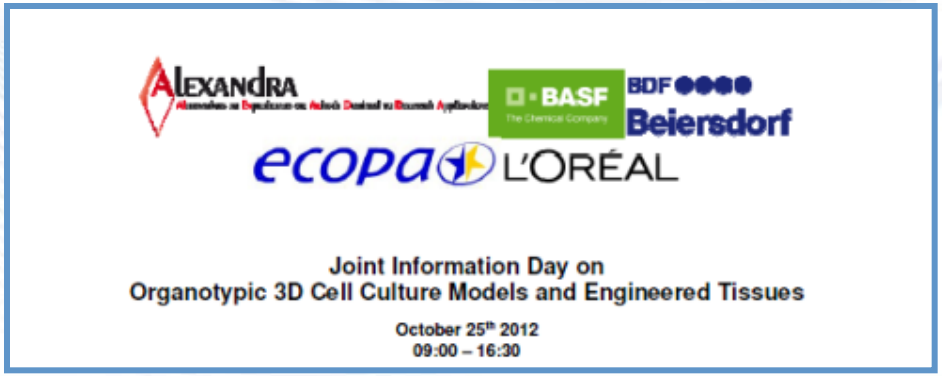






How can the cell system fit onto a platform, and what platform schematic would work best?

- **After aggregation the 3D culture can be transferred to most platforms**
- **Without rotation stable for at least 72 hours (LUHMES and rat model)**
- **MEA platforms - neuronal functionality (electrical activity recording)**
- **Larger samples size for e.g. intracellular metabolomics and transcriptomics analysis**



3D vs. 2D cultures

- Increased cell survival
- Increased differentiation
- Increased cell – cell interaction
- Reproducing better the complexity of the organ
- Endpoints need optimization
- More complex – lower reproducibility

Toxicology - $3 billion of testing to regulate $10 trillion of trade

Human-on-chip

Problems

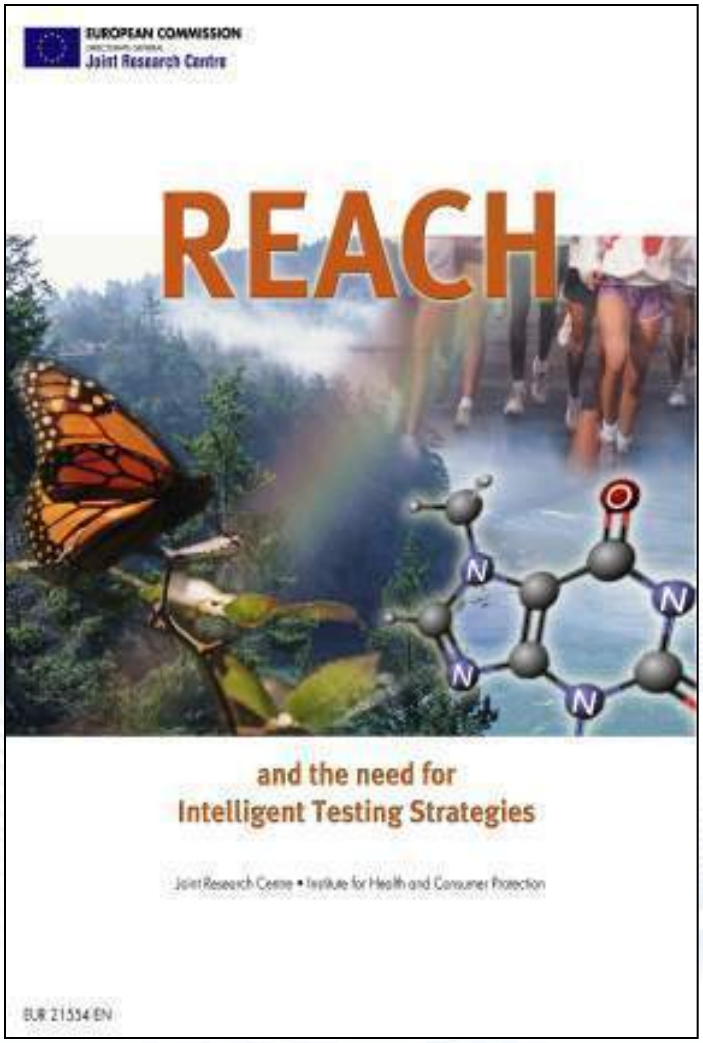
- **Throughput** −
- **Costs** −
- **Predictivity** + ?
- **Too precautionary** ?
- **Animal use** +
- **New products** +
- **New hazards** +
- **Mixtures** −
- **Individuals** +

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

US Stakeholder Forum 30-31 May 2013 in preparation (M. Stephens)

Looking for further interested partners!!!

Integrated Testing Strategies



- **Key contribution to REACH implementation process**
- **Use of different informations, not stand-alone replacement**

Just became available (AltWeb or ALTEX website)

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

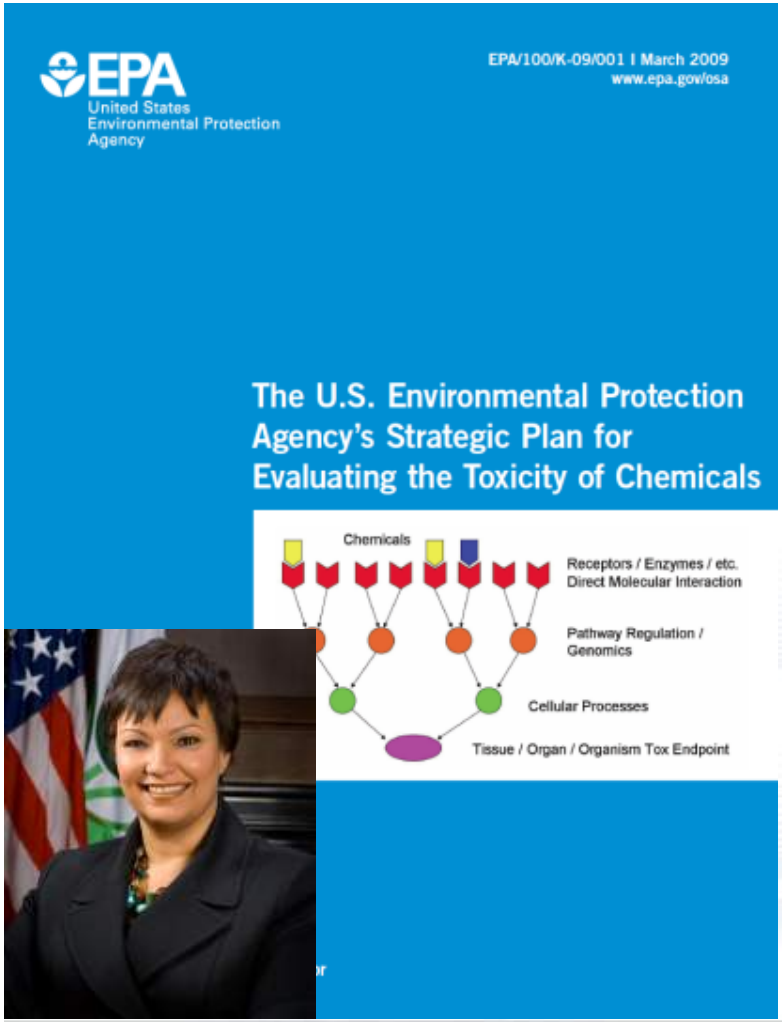
WoE, EBT, ITS…. Similar problems, but not the same

all: quality and data integration problem

EBT/WoE retrospective -- ITS prospective

WoE pragmatic -- EBT / ITS formalized

NAS vision report Tox-21c



"With an advanced field of regulatory science, new tools, including functional genomics, proteomics, metabolomics, high-throughput screening, and systems biology, we can replace current toxicology assays with tests that incorporate the mechanistic underpinnings of disease and of underlying toxic side effects." **M.A. Hamburg, FDA 2011**

"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"
F. Collins, NIH, 2008

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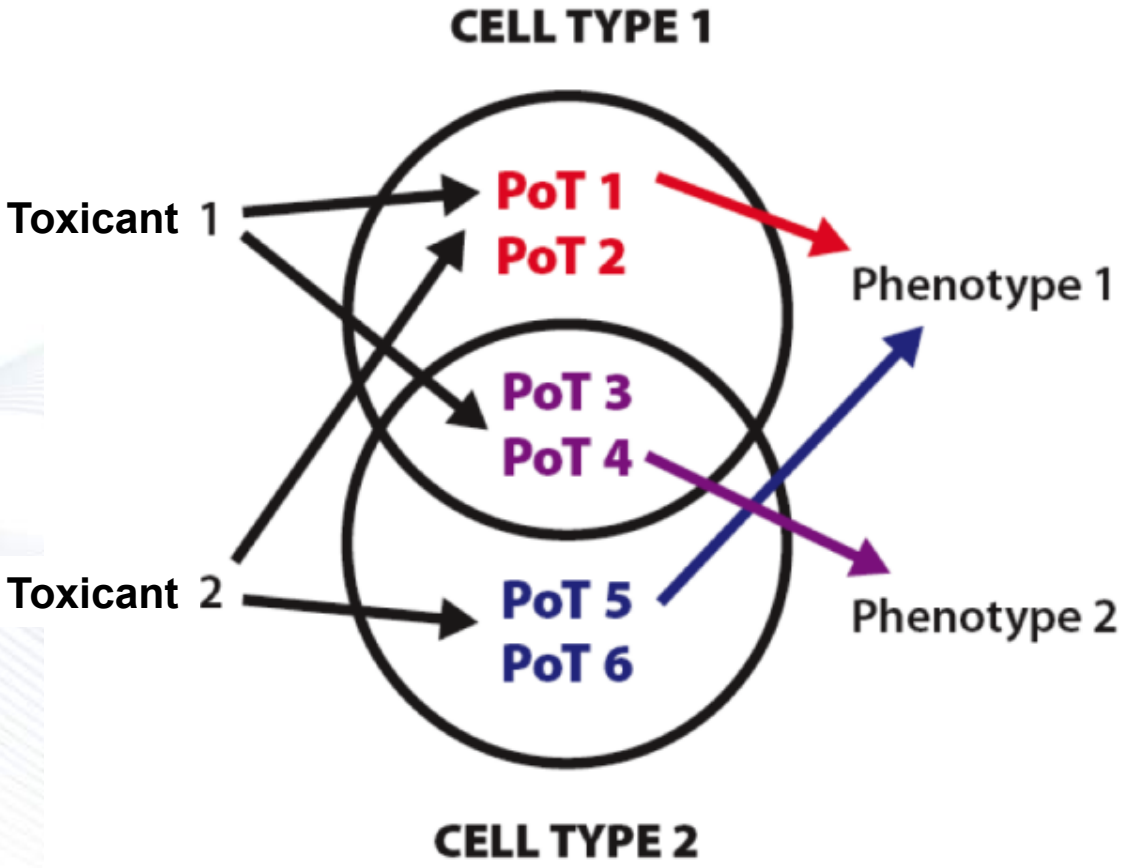
Initiatives implementing Tox-21c

Organization	Approach	Purpose	Outcome
US EPA & Tox21 (ToxCast Program)	High-throughput testing	Chemical prioritization (initially)	"Biological signatures"
Hamner Institute	Case studies	"Just do it"	Proof-of-principle
NIH project (CAAT-US)	Pathway mapping	Pathway ID & annotation	Human Toxome

The concept of (finite number of) pathways of toxicity



Annotation to:

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

Comprehensive list (Human Toxome) → **Negatives**

Johns Hopkins University Center for Alternatives to Animal Testing

OECD
ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT

PROPOSAL FOR A TEMPLATE, AND GUIDANCE ON DEVELOPING AND ASSESSING THE COMPLETENESS OF ADVERSE OUTCOME PATHWAYS

2012

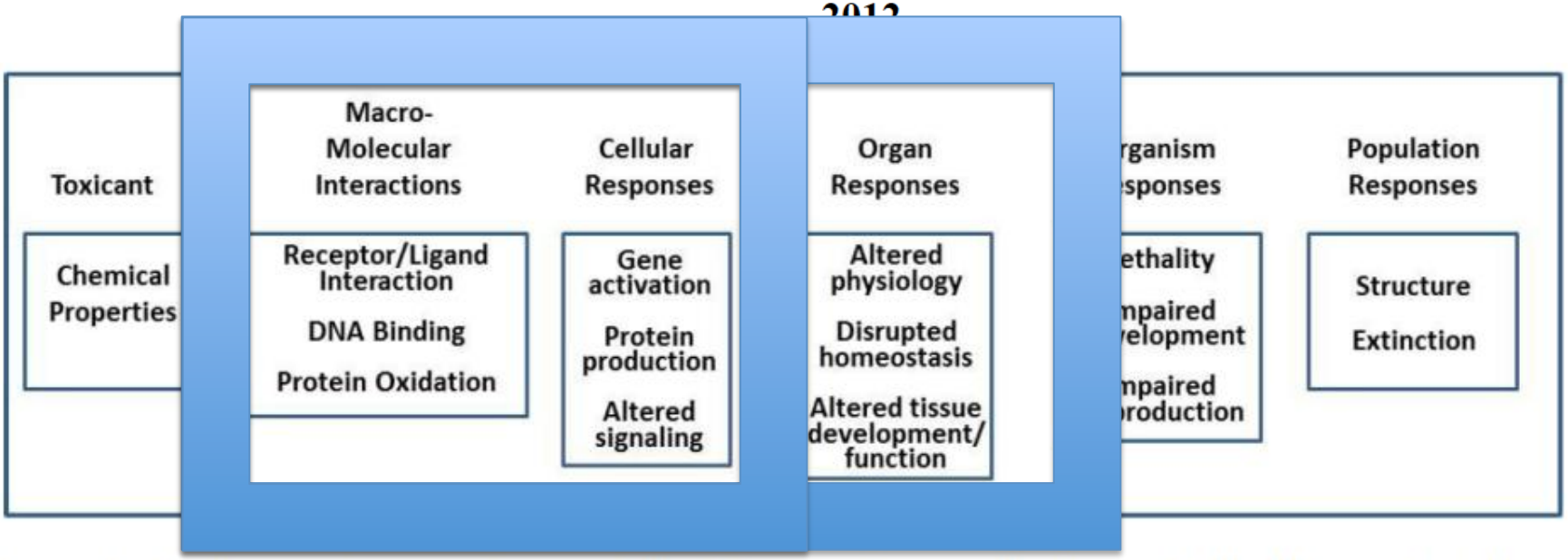


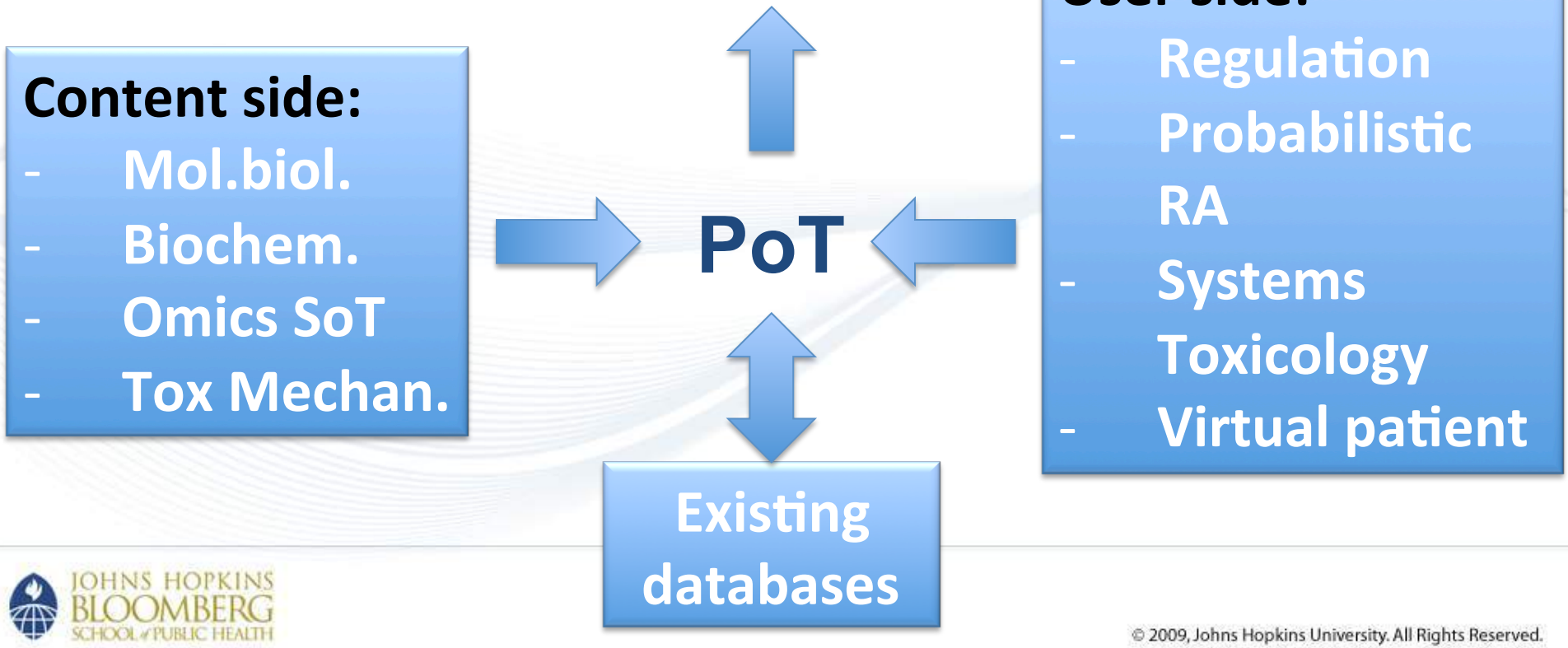
Figure1. A schematic representation of the Adverse Outcome Pathway (AOP) illustrated with reference to a number of pathways.

PoT

JOHNS HOPKINS BLOOMBERG SCHOOL of PUBLIC HEALTH

Workshop on the Concept and Tools for Pathways of Toxicity
October 10 -12, 2012, Baltimore, MD

Human Toxome database



PoToMaC - The Pathways of Toxicty Mapping Center

European branch?

Transformative Research Grant:

Mapping the Human Toxome by Systems Toxicology

HUMAN TOXICOLOGY PROJECT CONSORTIUM

7 companies, 3 stakeholders

2 Mar 2012

"Driven both by legislative mandate and scientific need, a new suite of in vitro and cell culture-based animal-free methods are gaining a foothold in toxicology labs."

LIFE SCIENCE TECHNOLOGIES

Produced by the *Science*/AAAS Custom Publishing Office

Toxicology

Animal-Free Toxicology

Sometimes, in Vitro is Better

The next time you use shampoo, air freshener, or moisturizing cream, consider this: How do you know it's safe? In all likelihood, whatever toxicologic screening its component ingredients were subjected to involved laboratory animals, the method of choice for decades and the industry's reigning "gold standard." Yet as Bob Dylan once put it, the times, they are a-changing. Animal-based testing is expensive and time-consuming, morally and ethically troubling, and most significantly, often a poor predictor of human toxicity. Animals aren't going anywhere just yet. But their numbers are dropping. Driven both by legislative mandate and scientific need, a new suite of in vitro and cell culture-based animal-free methods are gaining a foothold in toxicology labs. **By Jeffrey M. Perkel**

One key player in the modernization of toxicology screening is automation.

ALTEX 24 (2007) 67-73

Thomas Hartung, ECVAM

Food for Thought ... on Validation

View Online / Journal Homepage
Dynamic Article Links

Toxicology Research

Cite this: DOI: 10.1039/c2tx20011b

www.rsc.org/tx

REVIEW

Validation and quality control of replacement alternatives – current status and future challenges

Marcel Leist, Nina Hasiwa, Mardas Daneshian and Thomas Hartung

Received 5th February 2012, Accepted 9th May 2012
DOI: 10.1039/c2tx20011b

Definition of Validation

ALTEX 27 (2010) 253-263

Evidence-Based Toxicology – the Toolbox of Validation for the 21st Century?

Thomas Hartung
Johns Hopkins University, Bloomberg School of Public Health, Dept. Environmental Health Sciences, Center for Alternatives to Animal Testing (CAAT), Doerenkamp-Zbinden Chair for Evidence-based Toxicology, Baltimore, MD, USA, and Professor of Pharmacology and Toxicology, University of Konstanz, Germany

Knowledge:
- PoT
- MoA

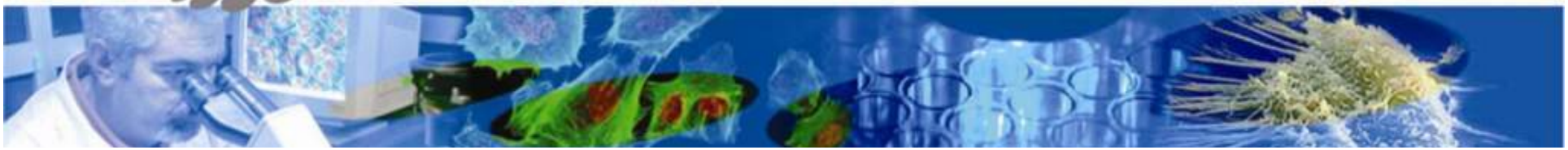
Mechanistic instead of correlative validation

1. Identify PoT (AOP, MoA…)
2. Include in Human Toxome knowledge base
   Governance? EBT?
3. Design PoT-based reproducible assays
4. Show PoT coverage with well established
   reference chemicals EBT?
5. Include in ITS

6. Food for Thought article in ALTEX in press

EBT

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



Evidence-based Toxicology

"Evidence-based medicine goes toxicology!"

Hoffmann and Hartung "Toward an evidence-based toxicology", Human Exp. Tox., 2006

Mar 2011: US EBTC
Oct 2011: Secretariat at CAAT
Jan 2012: First conference hosted by EPA

Kick-off meeting of the Evidence-Based Toxicology Collaboration (EBTC) Europe

ebtc
Evidence-based Toxicology Collaboration

In conjunction with Eurotox Congress 2012 (Stockholm, Sweden)

June 17, 2012
15:30h - 17:30h

Radisson Blu Royal Viking Hotel • Vasagatan 1, Stockholm, Sweden
Complimentary Registration: http://www.ebtox.com

Just became available (AltWeb or ALTEX website)

Workshop Report

Evidence-based Toxicology for the 21st Century: Opportunities and Challenges*

Martin L. Stephens [1], Melvin Andersen [2], Richard A. Becker [3], Kellyn Betts [4], Kim Boekelheide [5], Ed Carney [6], Robert Chapin [7], Dennis Devlin [8], Suzanne Fitzpatrick [9], John R. Fowle III [10], Patricia Harlow [11], Thomas Hartung [1], Sebastian Hoffmann [12], Michael Holsapple [13], Abigail Jacobs [11], Richard Judson [14], Olga Naidenko [15], Tim Pastoor [16], Grace Patlewicz [17], Andrew Rowan [18], Roberta Scherer [1], Rashid Shaikh [19], Ted Simon [20], Douglas Wolf [14], and Joanne Zurlo [1]

Perspectives on Validation of High-Throughput Assays Supporting 21st Century Toxicity Testing

Richard Judson [1], Robert Kavlock [1], Matthew Martin [1], David Reif [1], Keith Houck [1], Thomas Knudsen [1], Ann Richard [1], Raymond R. Tice [2], Maurice Whelan [3], Menghang Xia [4], Ruili Huang [4], Christopher Austin [4], George Daston [5], Thomas Hartung [6], John R. Fowle III [7], William Wooge [8], Weida Tong [9], and David Dix [1]

Johns Hopkins University Center for Alternatives to Animal Testing

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Evidence-based Toxicology Collaboration

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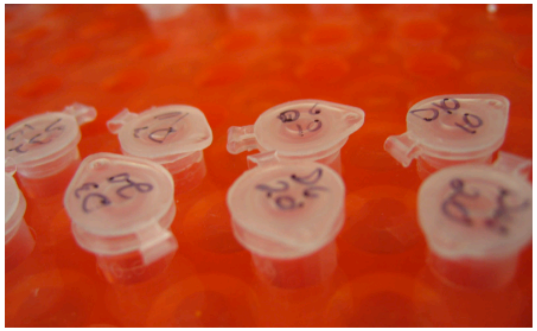
What is Evidence-based Toxicology?

The Evidence-Based Toxicology (EBT) Collaboration has recently taken up the challenge of translating evidence-based approaches from medicine to toxicology. The Collaboration has closely coordinated steering committees in the US and Europe with members drawn from government agencies, academia, and industry. More. . . .



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The US EBTC Steering Committee held an informal tutorial on systematic reviews (SRs) on July 23, 2012 at Johns Hopkins S...

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In conjunction with Eurotox Congress 2012 (Stockholm, Sweden) June 17, 2012 | 15:30h - 17:30h Radisson Blu Royal Vi...

The difficulty lies, not in the new ideas, but in escaping from the old ones.

John Maynard Keynes

(1883 - 1946)