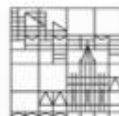


# *Roadmap for animal-free drug testing*

**Thomas Hartung, Marcel Leist  
& CAAT team**



## *Two sides of one coin...*

**Animal  
Welfare**

**3Rs**



**Improved  
Safety  
Sciences  
& Drug  
Development**



**Shortcomings of animal tests**

## Scientific American 2005

# PROTECTING MORE THAN **ANIMALS**

Reducing animal suffering often has the unexpected benefit of yielding more **RIGOROUS SAFETY TESTS**

By Alan M. Goldberg and  
Thomas Hartung



- **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**

Johns Hopkins University  
**Center for Alternatives  
to Animal Testing**

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**Pharma Reduction &  
Refinement Work Group  
(Joanne Zurlo)  
EU branch 26 Oct 12  
Awards**

**2013 EU mtg hosted by  
Roche / Novartis, FDA  
mtg. Reg. Tox,  
NIH Social Housing**

**Symposium on Social Housing of Laboratory Animals  
October 5-6, 2014 • University of Colorado Anschutz  
Medical Campus with NIH, APHIS and AWIC (USDA)**



DOERENKAMP-ZBINDEN  
Foundation



**32 articles / reports published**

**2 commissioned articles in preparation**

**5 workshop reports pending**

**In vitro publication standards**

**5+ workshops planned**

## *Ambassadors*

**Bas Blaauboer**

**Alan Goldberg**

**Thomas Hartung**

**Marcel Leist**





Brussels, March 2012

DC, May 30-31, 2013



**Stakeholder Fora**



**A Roadmap for the Development  
of Alternative (Non-Animal) Methods  
for Systemic Toxicity Testing**



## Alternatives Research & Development FOUNDATION



National Institute of  
Environmental Health Sciences

# Workshop and Infoday on MPS 8-11 June, Berlin



Joint Information Day on

„Biology inspired microphysiological systems – status and future“

June 11th 2015  
09:00 – 16:30

SANA Hotel (<http://bit.ly/16n7tQN>), Berlin, Germany

And more to come:

- **GCCP for iPSC,**  
**Baltimore, 1-3 June**
- **Adversity *in vitro*,**  
**Providence, 25-26 June**
- **Endocrine disruptors,**  
**Ranco, Italy, 20-22 July**
- **Cell Supply, Konstanz,**  
**30 Nov – 2 Dec**

**Average cost**  
~~\$1.4 billion~~ **\$4-11 billion**  
**Forbes 2012**

**95% fail**  
**(Arrowsmith 2012)**

~~99% fail:~~  
**20% tox not predicted**  
**- 40% no efficacy**



*Research*



*Drug development*

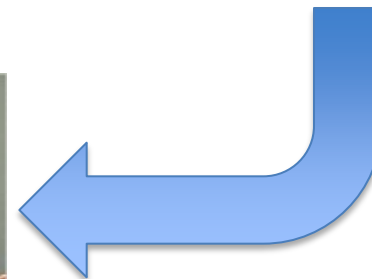


*Clinical trials*

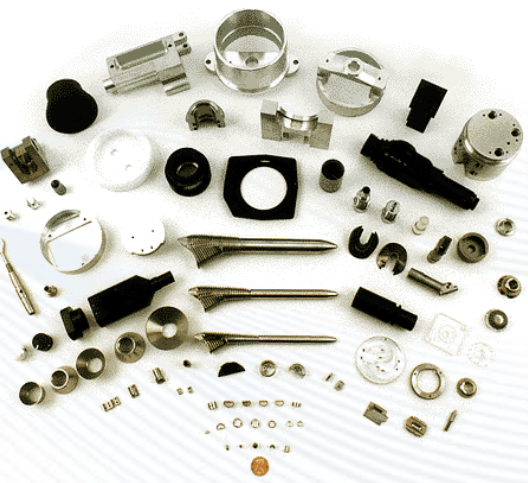
**1 in 100 patients**  
**in hospitals dies**  
**from adverse**  
**drug reactions**



**47 drugs**  
**withdrawn**  
**since 1990**



# Challenging new products



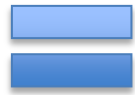
- **Biologicals** (biopharmaceutical products)
- **Cell therapies**
- **Genetically modified and functional food (nutraceuticals)**
- **Medical countermeasures to biological and chemical terrorism and warfare agents**
- **Medical devices**
- **Nanoparticles**



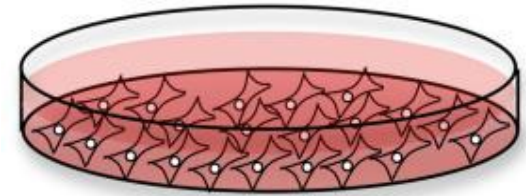
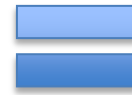
# All models are wrong, some are useful (G. Box)



naive



not less naive



60%

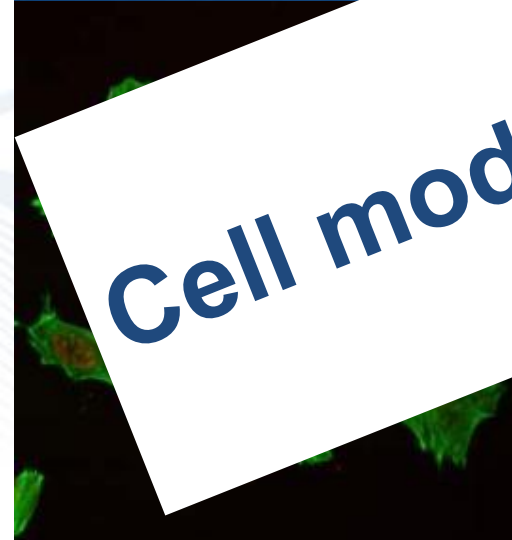


## *Limitations of in vitro models*

- Mycoplasma
- Dedifferentiation
- growth

**Cell models have not less limitations**

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



# Workshop on GCCP for iPSC 1-3 June, Baltimore



## 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)

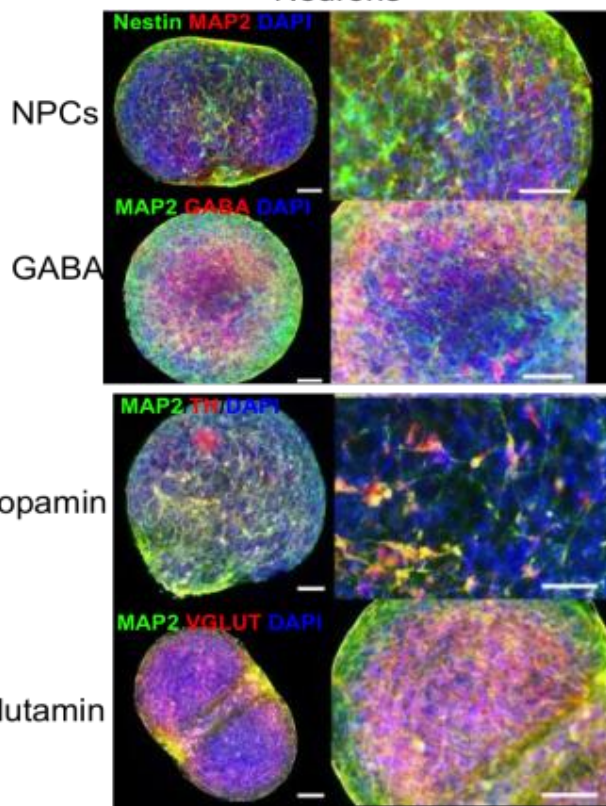
**Structure / Activity-  
Relationships**  
(Correlations)

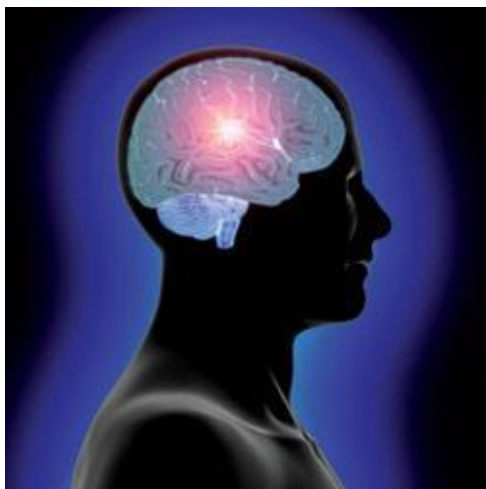


## *Human “mini-brain” developing from iPSC*

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

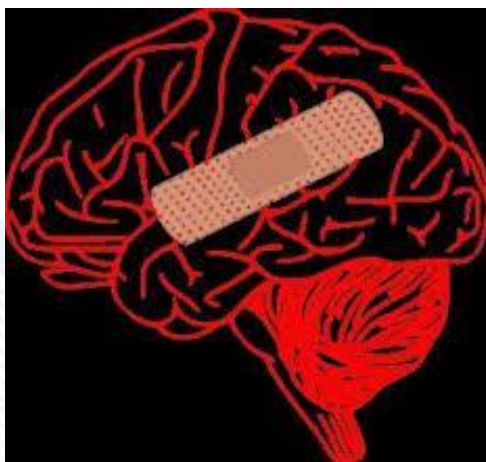
Neurons





## *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



## Early Alternatives

**Cell Culture**  
(one cell type,  
few parameters)

## Today

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

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

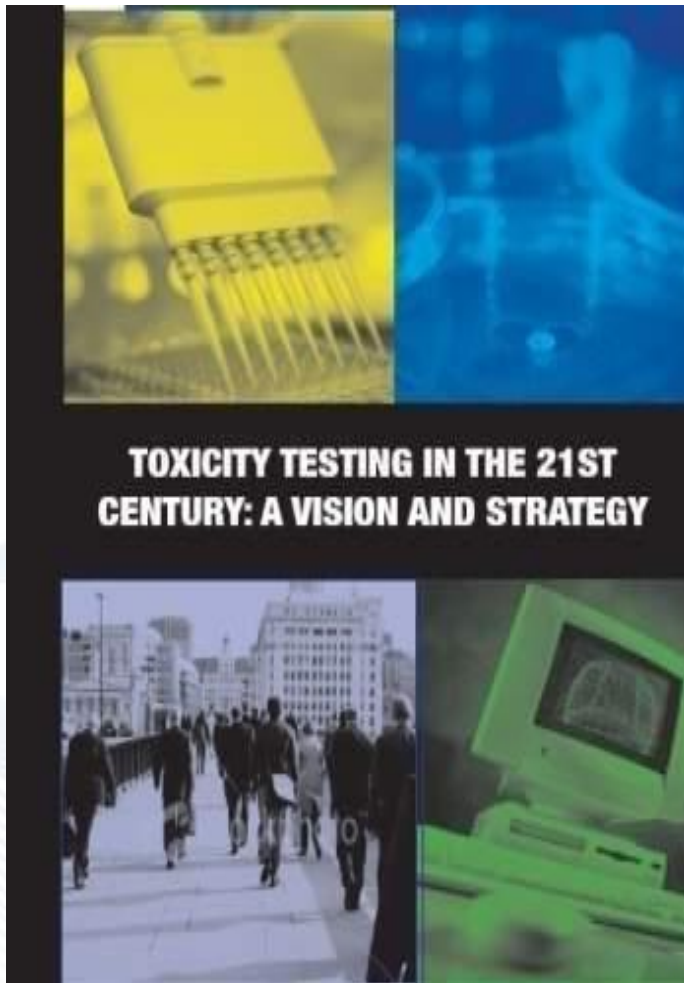
**Automated Cell  
Culture** (high-  
throughput Screening)

## Future

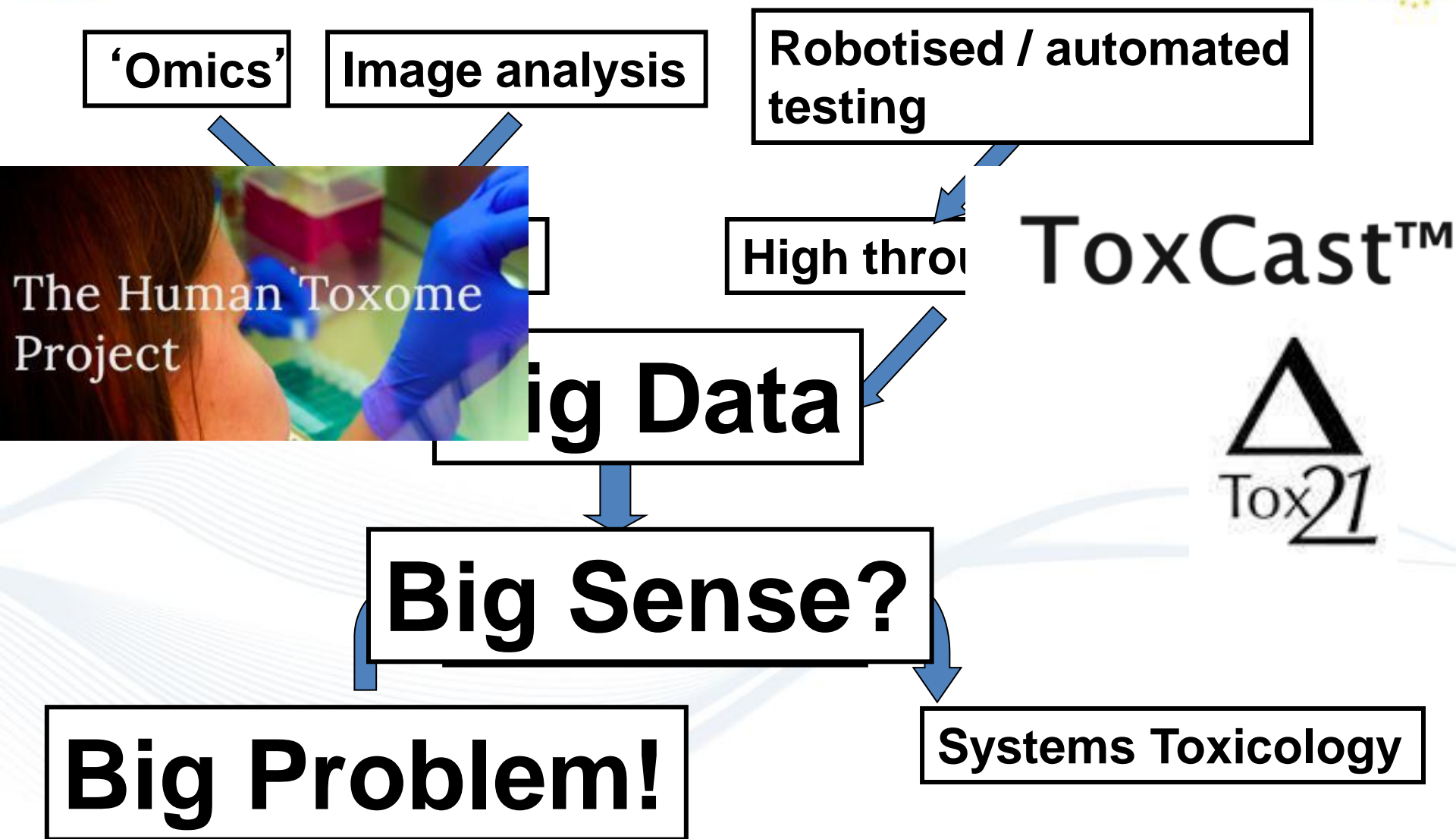
**Human-on-chip**  
(Multi-Organ Models  
With Microfluidics)

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


**Structure / Activity-  
Relationships**  
(Correlations)



# Mechanistic & evidence-based toxicology



The Human Toxome Project



---

Believe it or not: how much can we rely on published data on potential drug targets?

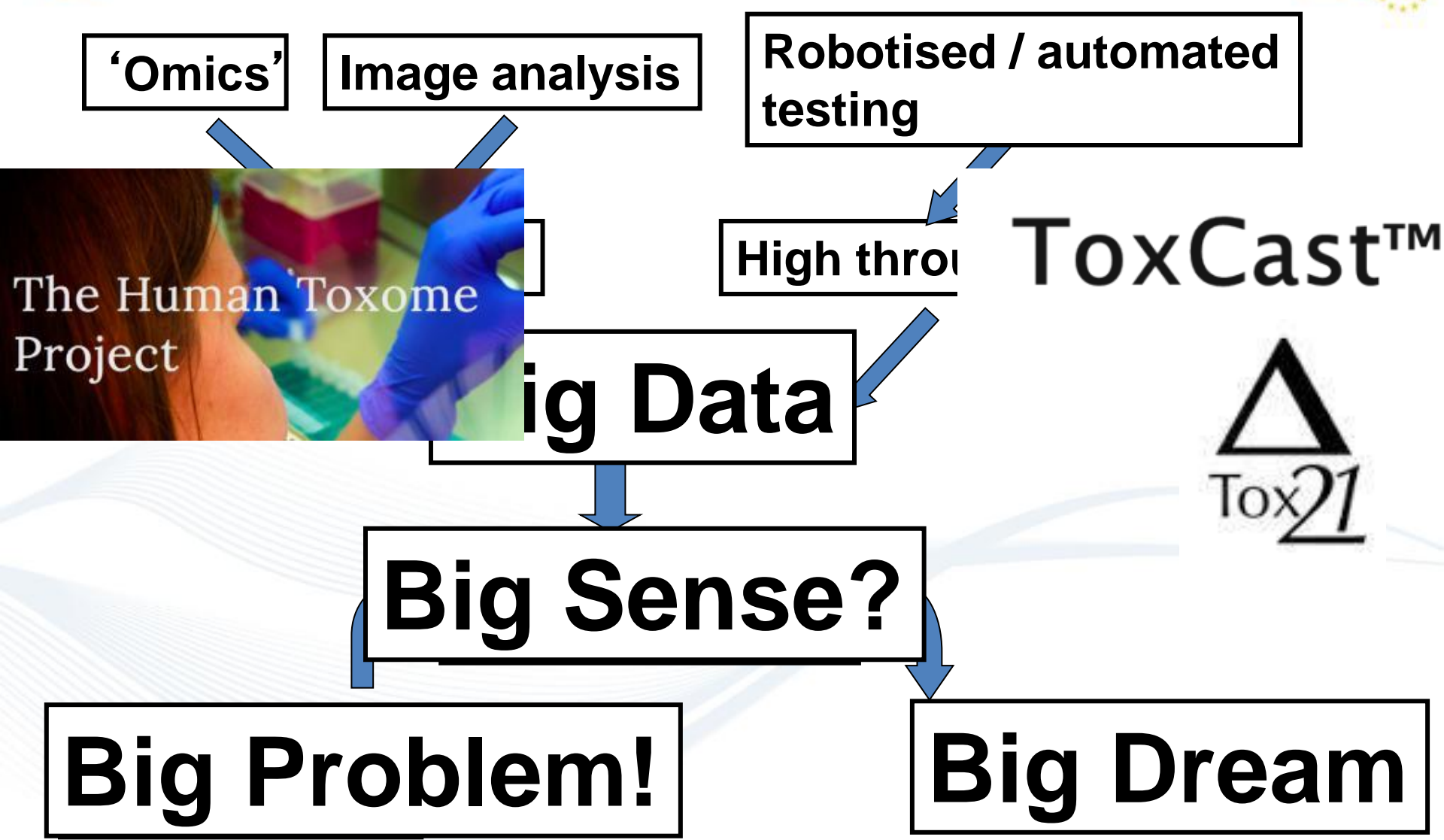
**This is why I do not believe in using existing knowledge without systematic review to form a point of reference**

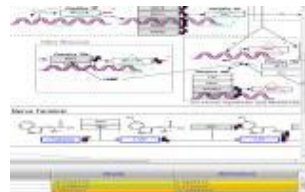
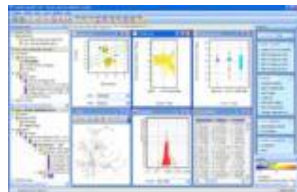
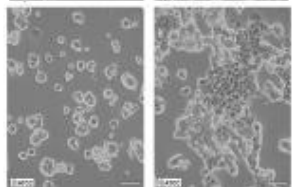
**Importance of untargeted approaches**

**cases, resulted in termination of the projects**

**NATURE REVIEWS | DRUG DISCOVERY**

*We need less research, better research, and research done for the right reasons*





**In vitro  
model**

**omics data  
generation**

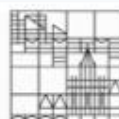
**Software  
tools**

**Pathways  
of Toxicity**

**Validation  
tools**

**Human  
Toxome  
Database**

# Mapping the Human Toxome by Systems Toxicology



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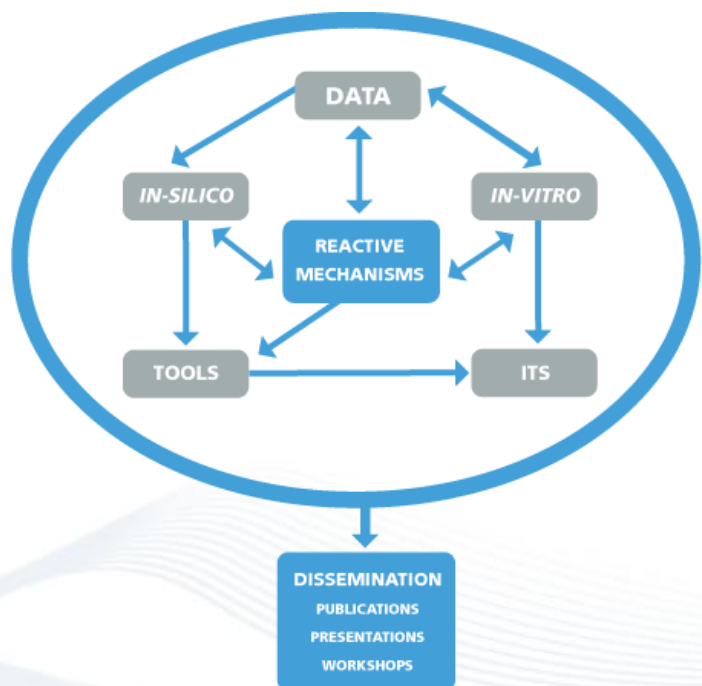
**Automated Cell  
Culture** (high-  
throughput Screening)

**Structure / Activity-  
Relationships**  
(Correlations)

**Integrated  
Test Strategies**  
(combined tests)

**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**

The Future  
NEXT EXIT

# Food for Thought ... Integrated Testing Strategies for Safety Assessments

*Thomas Hartung<sup>1,2</sup>, Tom Luechtefeld<sup>1</sup>, Alexandra Maertens<sup>1</sup>, and Andre Kleensang<sup>1</sup>*

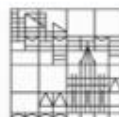
<sup>1</sup>Johns Hopkins University, Bloomberg School of Public Health, CAAT, Baltimore, USA; <sup>2</sup>University of Konstanz, CAAT-Europe, Germany



## t4 Workshop Report\*

# Integrated Testing Strategies (ITS) for Safety Assessment

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



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## *The gift from validation to life sciences*

**Validation of alternative tests is one of the rare examples of quality assurance in biomedical research (relevance, not only reproducibility)**

**“Evidence-based medicine goes in vitro!”**

**OECD guidance document, how to apply Good Laboratory Practice in vitro**

**Good Cell Culture Practice (minimal standards for academia)**

**Good Validation Practice (jointly with OECD, ICCVAM, JaCVAM)**

**Evidence-based Toxicology Collaboration (US & EU)**

2006-7: Publication / 1<sup>st</sup> conference

Mar 2011: US EBTC

Oct 2011: Secretariat at CAAT

[www.ebtox.com](http://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

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



**EBT.**

# Systematic review & related approaches: Gaining acceptance in toxicology



## A different application of systematic reviews: **Assessing Test Method Performance**

### EBTC Project (in progress):

*How well does Zebrafish Embryo Testing (ZET) predict the presence or absence of malformations in studies of pre-natal development toxicity in rats and rabbits (OECD TG 414 and equivalents)?*



**Review quality scoring tools and handbook systematic reviews in preparation**



## New organization in progress:

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

## New BoD Members

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

Ex officio, non-voting:

Sebastian Hoffmann & Martin Stephens

# Which R of the 3?



## The 4<sup>th</sup> R?

**Read-across**

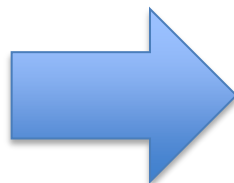
**Refine**

**Replace**

**Reduce\***

**\*pesticides**





**Alternative approaches have become one of the most dynamic areas of toxicology – “3Rs Plus, not 1R”**

- **Embracing latest technologies**
- **Emerging new concepts**
- **International harmonization and collaboration**
- **Spanning cross industrial sectors**
- **A role model for all life sciences as to quality assurance, assessment of predictive capacity and humane science**

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

**John Maynard Keynes**

**(1883 - 1946)**

