The maturing toolbox of toxicology for the 21st century

Thomas Hartung
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
Funding from industry, philanthropy and research funding agencies

The Bernice Barbour Foundation

...and individuals
32 articles / reports published

2 commissioned articles in preparation

5 workshop reports pending

In vitro publication standards

5+ workshops planned

**Ambassadors**

Bas Blaauuboer
Alan Goldberg
Thomas Hartung
Marcel Leist
**Early Alternatives**

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

**Today**

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

**Future**

*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
Opportunities for human mini-brain research

- Map the neurotoxic chemical universe
- Characterization of medical countermeasures
- Neurotoxic and DNTToxic 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
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Today

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

Automated Cell Culture
(high-throughput Screening)

Future

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Toxicity Mechanisms
(“Adverse Outcome Pathways”, “Human Toxome”)
'Omics'  Image analysis  Robotised / automated testing

Big Data  High throughput

Big Sense?

Big Problem!

ToxCast™  Tox21

Systems Toxicology

The Human Toxome Project

Information rich

Bioinformatics & Data mining

Knowledge on pathways

High content

Robotised / automated testing

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Big Data

Big Sense?

Big Problem!
Fifty-three papers were deemed ‘landmark’ studies … scientific findings were confirmed in only 6 (11%) cases. Even knowing the limitations of preclinical research, this was a shocking result.

… data from 67 projects, … This analysis revealed that only in ~20–25% of the projects were the relevant published data completely in line with our in-house findings... In almost two-thirds of the projects, there were inconsistencies between published data and in-house data that either considerably prolonged the duration of the target validation process or, in most cases, resulted in termination of the projects.

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

Importance of untargeted approaches.

We need less research, better research, and research done for the right reasons.
‘Omics’ Image analysis High throughput

Robotised / automated testing

High throughput

Big Data

Big Sense?

The Human Toxome Project

Big Problem!

Big Dream

ToxCast™

Tox21
Mapping the Human Toxome by Systems Toxicology
(Pre-)Validated work

- Robust protocols, good cell models
- Regulatory acceptance available or in progress
- Available reference substances
- Thresholds of adversity defined

MCF-7 cells (pre-validated by ICCVAM) &
- Initial set of endocrine disrupting chemicals selected from a priority list of 53 reference compounds identified by ICCVAM

Karyotyping
<table>
<thead>
<tr>
<th>Success</th>
<th>Failure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Consortium with intense interactions</td>
<td>No link to high-throughput testing</td>
</tr>
<tr>
<td>PoT concept furthered, mechanistic validation</td>
<td>No annotation yet</td>
</tr>
<tr>
<td>Improved cell system standardization</td>
<td>MCF-7 not really suitable</td>
</tr>
<tr>
<td>Some problems omics overcome, QA</td>
<td>Metabolite identification</td>
</tr>
<tr>
<td>Cloud Server, Toxome Collaboratorium, GeneSpring expansion and other tools</td>
<td>No real PoT identification yet</td>
</tr>
<tr>
<td>High visibility</td>
<td>No expansion to other hazards or regions</td>
</tr>
<tr>
<td>Workflow established (integrated omics analysis, weighted gene connection network analysis, transcription factor analysis and systematic literature mining)</td>
<td>PoT #1 emerging</td>
</tr>
</tbody>
</table>
What this project is not....

...an endocrine disruptor screening project

...a test development project

...an academic publish or perish project

...a five year project
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(Correlations)

Modeling
(Receptor binding, Virtual Organs, Kinetics)

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Automated Cell Culture
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Cell Culture + Omics or Image Analysis
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Toxicity Mechanisms
(“Adverse Outcome Pathways”, “Human Toxome”)

Human-on-chip
(Multi-Organ Models With Microfluidics)
transatlantic think tank for toxicology

"The difficulty lies, not in the new ideas, but in escaping from the old ones."
John Maynard Keynes (1883-1946)

"This report, by its very length, defends itself against the risk of being read."
Winston Churchill (1874-1965)

t4 Report*


David A. Baskettet, Harvey Clewell, Ian Kimber, Annamaria Rossi, Bas Blaauboer, Robert Burrier, Mardas Daneshian, Chantra Eskes, Alan Goldberg, Nina Hasiwa, Sebastian Hoffmann, Joanna Jaworska, Thomas B. Knudsen, Robert Landsiedel, Marcel Leist, Paul Locke, Gavin Maxwell, James McKim, Emily A. McVey, Gladys Ouédraogo, Grace Patlewicz, Olavi Pelkonen, Erwin Roggen, Costanza Rovida, Irmela Ruhdel, Michael Schwarz, Andreas Schepky, Greet Schoeters, Nigel Skinner, Kerstin Trentz, Marian Turner, Philippe Vanparys, James Yager, Joanne Zurlo, and Thomas Hartung

*Contributors and institutions are listed in the Acknowledgments section.
Toxicokinetics

- The necessary complement to ALL in vitro approaches -> case studies

- In silico approaches to be optimized: PBPK modeling platforms → user-friendly; open source; physiol parameters (dermal, inhalation exposure, ..)
- Data collection for QSPR modeling and simulation: metabolism, distribution, protein binding, .... Simulation based on PhysChem principles
- Need for data collections to support IVIVE: incorporation of in vitro data (barrier models (placenta, mammary, testis, intestine, brain, ..), transporters (!))
- Problems for bioavailability, urinary excretion, non-hepatic metabolism, BBB, GI metabolism,
- Free concentrations/target concentrations in vitro
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**Integrated Test Strategies**
(combined tests)

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(Receptor binding, Virtual Organs, Kinetics)

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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 Hartung¹,², Tom Luechtfeld¹, Alexandra Maertens¹, and Andre Kleensang¹
¹Johns Hopkins University, Bloomberg School of Public Health, CAAT, Baltimore, USA; ²University of Konstanz, CAAT-Europe, Germany

transatlantic think tank for toxicology

t⁴ Workshop Report*
Integrated Testing Strategies (ITS) for Safety Assessment

Costanza Rovida¹, Nathalie Alépée², Anne M. Api³, David A. Basketter⁴, Frédéric Y. Bois⁵, Francesca Caloni⁶, Emanuela Corsini⁷, Mardas Daneshian¹, Chantra Eskes⁸, Janine Ezendam⁹, Horst Fuchs¹⁰, Patrick Hayden¹¹, Christa Hegele-Hartung¹², Sebastian Hoffmann¹³, Bruno Hubesch¹⁴, Miriam N. Jacobs¹⁵, Joanna Jaworska¹⁶, André Kleensang²⁰, Nicole Kleinstreuer¹⁷, Jon Lalko³, Robert Landsiedel¹⁸, Frédéric Lebreux¹⁹, Thomas Luechtfeld²⁰, Monica Locatelli²¹, Annette Mehling¹⁸, Andreas Natsch²², Jonathan W. Pitchford²³, Donald Prater²⁴, Pilar Prieto²⁵, Andreas Schepky²⁶, Gerrit Schüürmann²⁷,²⁸, Lena Smirnova²⁰, Colleen Toole²⁹, Erwin van Vliet³⁰, Dirk Weisensee¹⁰ and Thomas Hartung¹,²,²⁰
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Future

- **Human-on-chip** (Multi-Organ Models With Microfluidics)
- **Toxicity Mechanisms** (“Adverse Outcome Pathways”, “Human Toxome”)
- **Systems Toxicology** (“Virtual Patient”)
Systems Biology / (Toxicology)

Glossary of Reference Terms for Alternative Test Methods and their Validation

Daniele Ferrario¹, Roberta Brustio² and Thomas Hartung³,⁴

Systems biology: Study of the mechanisms underlying complex biological processes as integrated systems of many diverse, interacting components. It involves (1) collection of large sets of experimental data (by high-throughput technologies and/or by mining the literature of reductionist molecular biology and biochemistry); (2) proposal of mathematical models that might account for at least some significant aspects of this data set; (3) accurate computer solution of the mathematical equations to obtain numerical predictions; and (4) assessment of the quality of the model by comparing numerical simulations with the experimental data (Duffus et al., 2007; id).

High-content, big data

Mining literature systematically

Modeling, virtual experiments
Emerging concepts

Article series in ALTEX

Food for Thought ... on Mapping the Human Toxome

Thomas Hartung and Mary McBride

1 CAAT, Johns Hopkins University, Bloomberg School of Public Health, Baltimore, MD, USA, and CAAT-Europe, University of Konstanz, Germany; 2 Agilent Technologies, Government Relations, Life Sciences and Chemical Analysis, Washington, DC, USA

Food for Thought ...


Melvin E. Anderson, Harvey J. Clewell, III, Paul L. Carmichael, and Kim Boekelheide

1 The Institute for Chemical Safety Sciences, The Hamner Institutes for Health Sciences, Research Triangle Park, NC, USA; 2 Department of Pathology and Laboratory Medicine, Brown University, Providence, RI, USA

Food for Thought ... on Systems Toxicology

Thomas Hartung, Erwin van Vliet, Joanna Jaworska, Leo Bonilla, Nigel Skinner, and Russell Thomas

1 Johns Hopkins University, Bloomberg School of Public Health, Center for Alternatives to Animal Testing (CAAT), Baltimore, USA and University of Konstanz, CAAT-Europe, Germany; 2 Hospital Clinic – Universitat de Barcelona, Department of Maternal-Fetal Medicine, Fetal and Perinatal Medicine Research Group, Barcelona, Spain; 3 Procter & Gamble, Brussels, Belgium; 4 Agilent Technologies, Inc., Santa Clara, CA, USA; 5 The Hamner Institutes for Health Sciences, Research Triangle Park, NC, USA

All online available
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.

InfoDays

Green Toxicology

Connecticut, Dec 2012

Baltimore, Nov 2013

Zurich, Switzerland

23 Oct 2014

SoT 2015, San Diego

EUROTOX 2016
Food for Thought ...
Green Toxicology

Alexandra Maertens¹, Nicholas Anastas³, Pamela J. Spencer⁴, Martin Stephens¹, Alan Goldberg¹ and Thomas Hartung¹,²

¹Johns Hopkins University, Bloomberg School of Public Health, CAAT, Baltimore, MD, USA; ²CAAT-Europe, University of Konstanz, Germany; ³EPA Region 1, Boston, MA, USA; ⁴The Dow Chemical Company, Midland, MI, USA

Green Toxicology

Regulatory Toxicology

Exposure-driven testing
Thresholds of Tox. Concern
Tox-21c and 3Rs

Substance Design
Benign Design by (Q)SAR and other in silico

Research & Development
Early testing in chemico, in vitro and lower organisms

Production
Clean and sustainable production; Occupational hazard ▼

Product Life Cycle
Exposure & waste ▼
Which R of the 3?

The 4\textsuperscript{th} R?

Read-across

Refine

Replace

Reduce* [*pesticides]
CAAT Read-across Initiative

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

Grace Patlewicz¹, Nicholas Ball², Richard A. Becker³, Ewan D. Booth⁴, Mark T. D. Cronin⁵, Dinant Kroese⁶, David Steup⁷, Ben van Ravenzwaay⁸ and Thomas Hartung⁹*

Read-across-21c

- Negative vs. positive read-across
- Support by biological data not only structure
- Expression of uncertainty
- Local validity
- Application to complex mixtures

“Test-across”, 2007
The difficulty lies, not in the new ideas, but in escaping from the old ones.

John Maynard Keynes
(1883 - 1946)