

New Approaches to Establishing Safety and Conducting Toxicology Studies

**Thomas Hartung & team
Center for Alternatives to Animal Testing**



Conflict of Interest Statement



ORGANOME

**Co-founder
- organoids**



**Green Chemistry
Advisory Panel**



**Consultant
Computational
Toxicology**



**Licensed
Pyrogen Test
Consultant**

Some problems of toxicology in pharma

*We are
not 70kg
rats !!!*

- **20% drug failure because of toxicity not predicted**
- **8% drugs withdrawn from market because of side-effects**
- **Occasional problems first in humans**
- **Information too late in process**
- **Poor prediction of drug interactions (killing 1:100 hospitalized patients)**
- **Sorting out of substances because of meaningless (animal and mutagenicity) tests**

Toxicology has the likely most reproducible animal tests (GLP, standardized protocols, some validated, high doses, substance effects in healthy animals) – their reproducibility:

- **Cancer bioassay: 57% (repeat or mouse vs. rat)**
- **Reproductive Tox: 60% between species**
- **Uterotrophic assay: 26% contradictory**
- **Skin sensitization: 77% guinea pig vs. mouse**
- **Severs eye irritation: 70% reproducible**
- **Acute fish tox: up to 6 log orders different**
- **Chronic tox: no correlation between mouse and rats or genders**

Irreprodu-*cell*-bility

Cell tests have not less problems!

- **Ca. 25% of cell lines misidentified**
- **15-25% mycoplasma infected**
- **Genetic instability**
- **Culture artifacts**

www.nature.com/scientificreports

SCIENTIFIC REPORTS 

OPEN

Genetic variability in a frozen batch of MCF-7 cells invisible in routine authentication affecting cell function

Received: 01 March 2016
Accepted: 13 June 2016
Published: 26 July 2016

Andre Kleensang¹, Marguerite M. Vantangol², Shelly Odwin-DaCosta³, Melvin E. Andersen³, Kim Boekelheide², Mounir Bouhifd¹, Albert J. Fornace Jr⁴, Heng-Hong Li⁵, Carolina B. Livi⁶, Samantha Madnick², Alexandra Maertens², Michael Rosenberg⁵, James D. Yager⁶, Liang Zhao¹ & Thomas Hartung^{1,7}

Pronounced genetic differences in frozen cells of the same lot from a cell bank

Kleensang (2016) Sci Rep 6, 28994

International Good Cell Culture Practice Collaboration



**EuroScience
Open Forum
Manchester 2016**

National Center



t⁴ workshop report

Good Cell Culture Practice for stem cells, stem-cell-derived models and organ-on-chip models

David Pamies¹, Anna Bal-Price², Anton Simeonov, Danilo Tagle, Dave Allen, David Gerhold, Dezhong Yin, Francesca Pistollato, Inutsuka Takahashi, Kevin G. Chen, Kristie Sullivan, Glynn Stacey, Harry Salem, Leist Marcel, Mardas Daneshian, Mohan Vemuri, Richard McFarland, Sandra Coecke², Suzanne C. Fitzpatrick, Tina Bahadori, Uma Lakshmipathy, Wen Bo Wang, Yuko Sekino, Kanda Yasunari, Thomas Hartung¹.

THE EUROPEAN UNION REFERENCE LABORATORY
FOR ALTERNATIVES TO ANIMAL TESTING

*Advancing Public Health
and Animal Welfare*

Evidence-based medicine goes toxicology

ToxSci Advance Access published May 5, 2016



SOT | Society of Toxicology
www.toxsci.oxfordjournals.org

TOXICOLOGICAL SCIENCES, 2016, 1-7

doi: 10.1093/toxsci/kfw059
Forum Article



FORUM ARTICLE

The Emergence of Systematic Review in Toxicology

Martin L. Stephens,^{a,1} Kellyn Betts,^b Nancy B. Beck,^c Vincent Cogliano,^d
Kay Dickersin,^e Suzanne Fitzpatrick,^f James Freeman,^g George Gray,^h
Thomas Hartung,^{a,1} Jennifer McPartland,^j Andrew A. Rooney,^k
Roberta W. Scherer,^e Didier Verloo,^l and Sebastian Hoffmann^m

www.ebtox.com

info@ebtox.com

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ELSEVIER

Contents lists available at ScienceDirect

Environment International

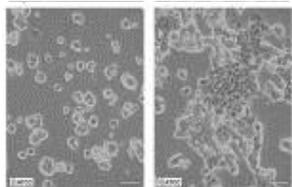
journal homepage: www.elsevier.com/locate/envint



Guidance on assessing the methodological and reporting quality of toxicologically relevant studies: A scoping review

Gbeminiyi O. Samuel^a, Sebastian Hoffmann^b, Robert A. Wright^c, Manoj Mathew Lalu^d, Grace Patlewicz^{e,1},
Richard A. Becker^f, George L. DeGeorge^g, Dean Fergusson^d, Thomas Hartung^a,
R. Jeffrey Lewis^h, Martin L. Stephens^{a,*}

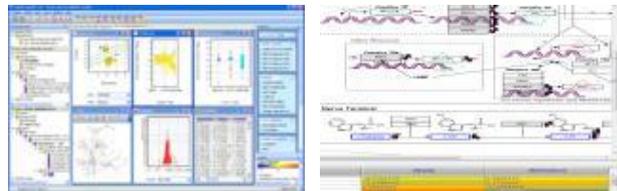
Pathways of toxicology from multi-omics



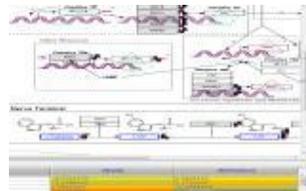
In vitro
model



omics data
generation



Software
tools



Pathways
of Toxicity



Validation
tools



Human
Toxome
Database

Mapping the Human Toxome by Systems Toxicology

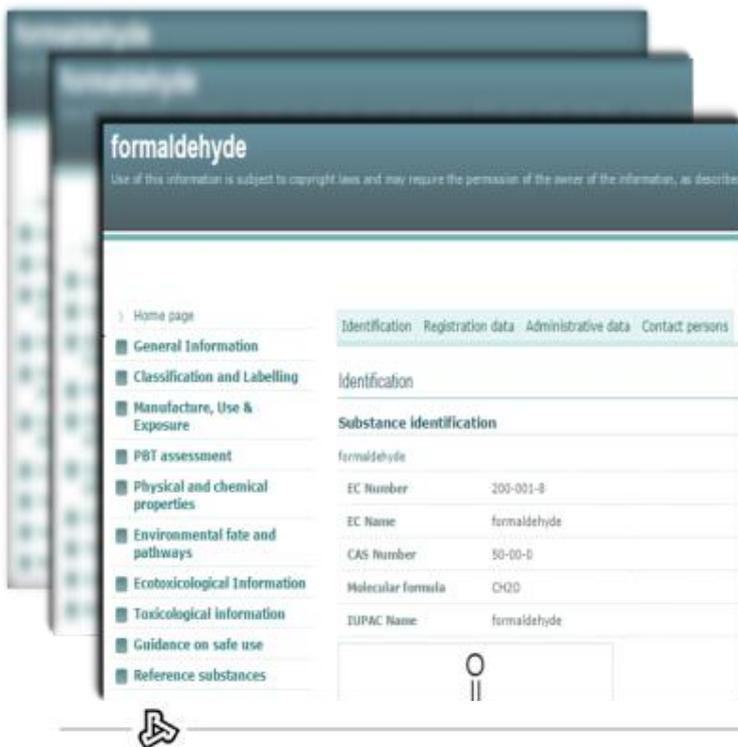
NIH Transformative Research Grant
15 publications, 2 under review
<http://humantoxome.com>



Mining REACH data

made machine-readable by natural language processing

10000 Dossiers Examined

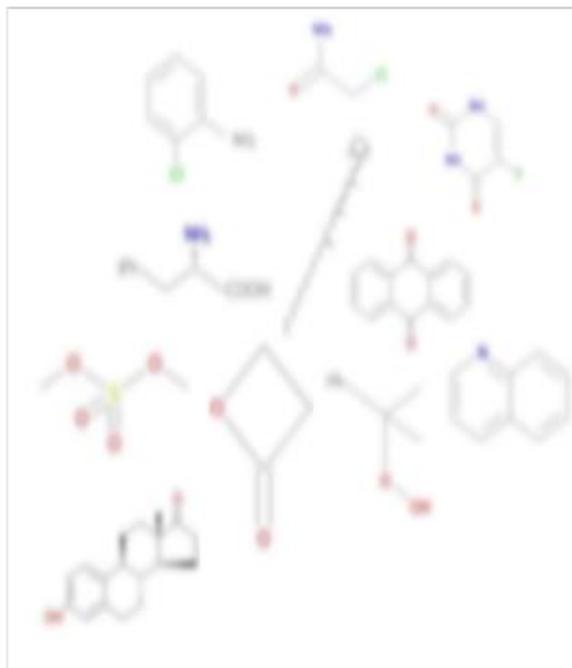


The image shows a screenshot of a REACH dossier for formaldehyde. The title is "formaldehyde" and there is a warning: "Use of this information is subject to copyright laws and may require the permission of the owner of the information, as described...". The left sidebar contains a navigation menu with categories: Home page, General Information, Classification and Labelling, Manufacture, Use & Exposure, PBT assessment, Physical and chemical properties, Environmental fate and pathways, Ecotoxicological Information, Toxicological Information, Guidance on safe use, and Reference substances. The main content area is divided into tabs: Identification, Registration data, Administrative data, and Contact persons. The "Identification" tab is active, showing the following information:

Identification	
Substance identification	
formaldehyde	
EC Number	200-001-8
EC Name	formaldehyde
CAS Number	50-00-0
Molecular formula	CH ₂ O
IUPAC Name	formaldehyde

Below the table is a chemical structure diagram of formaldehyde, showing a central carbon atom double-bonded to an oxygen atom and single-bonded to two hydrogen atoms.

9801 Chemicals



816000 Chemical Study Pairs

...
Formaldehyde-releasers: relationships to formaldehyde contac...
Textile dermatitis in patients with contact sensitization in...
Prospective study of clinical symptoms and skin test reactio...
Effect of Pyridine and Formaldehyde on a Macrophyte (Lemna m...
Acute toxicity of formaldehyde to the pearl oyster Pinctada ...
The effects of organic and inorganic pollutants on intracell...
Toxicity of Laundry Detergent Components to a Freshwater Cla...
Formalin: Its Toxicity to Nontarget Aquatic Organisms, Persi...
Befunde der Schadwirkung wassergefährdender Stoffe gegen Dap...
The Toxicity of Erythromycin, Minocycline, Malachite Green, ...
Low sensitivity of ciliary activity in the gills of Anodonta...
Cumulative toxicities of some inorganic salts to Daphnia mag...
Toxicity of selected chemicals to the Fairy shrimp, Streptoc...
Acute Toxicity of Formalin and Copper Sulfate to Striped Bas...
Survival of fish in 164 herbicides, insecticides, fungicides...
Use of formalin for selective control of tadpoles in the pre...
Effects of formalin treatment on the blood composition of sa...
Toxicity of 22 Therapeutic Compounds to Six Fishes
Toxicity of formalin to bullseye puffer fish (Sphoeroides an...
Studies on the Formalin Toxicity and Formaldehyde Residues i...
Evaluation of laboratory-made sludge for an anaerobic biodeg...
Experiences with the test scheme under the chemical control ...
Oxidative abilities of benzene-acclimated activated sludge
Anaerobic biodegradation of nitrogen-substituted and sulfona...
Effects of Formaldehyde on Cardiovascular System in In Situ ...
Contact and respiratory sensitizers can be identified by cyt...
Comparative assessment of toxicity of phenol, formaldehyde, ...
Grenzwerte der Schadwirkung wassergefährdender Stoffe gegen ...
Bioaccumulation and metabolic fate of pyrazolate and its hy...
Vergleichende toxikologische Befunde an Wasser-Bakterien
...

Research Article

Global Analysis of Publicly Available Safety Data for 9,801 Substances Registered under REACH from 2008-2014

Thomas Luechtefeld¹, Alexandra Maertens¹, Daniel P. Russo², Costanza Rovida⁴, Hao Zhu^{2,3} and Thomas Hartung^{1,4}

Largest toxicological database = “goldmine” for computational toxicology

CAAT Read-Across Collaboration

NEWS | IN DEPTH

TOXICOLOGY

A crystal ball for chemical safety

By comparing new chemicals to known compounds, toxicologists seek early hazard warnings

By Tania Rabesandratana

Every year, chemists invent thousands of new chemicals, and many ultimately find their way into global use. Predicting which ones will pose health or environmental hazards, however, has proven difficult. This week, a group of researchers unveiled a tool that could help streamline the process: a vast database of safety information that will allow users to compare new chemicals to existing compounds with similar structures, and flag potential risks.

"You could imagine that, before even synthesizing [a chemical], a chemist puts the structure into the [tool] to ask if it's safe," says toxicologist Thomas Hartung of the Johns Hopkins Bloomberg School of Public Health in Baltimore, Maryland, who led the effort.

Such predictive screening could help companies and government regulators reduce the need for lengthy, expensive animal testing, observers say, and identify safer alternatives to existing compounds. "We are very enthusiastic about what [Hartung's team] has done," says Tina Bahadori of the U.S. Environmental Protection Agency (EPA) in Washington, D.C., which has been expanding its own computational toxicology efforts. But Bahadori cautions that structural similarities, although promising, are just "one piece of the puzzle" in assessing a compound's safety.

To create the screening tool, which Hartung's team describes this week in *Alternatives to Animal Experiments*, the researchers dug deep into what he calls a "gold mine" of data collected by the European Chemicals Agency (ECHA). Under a 2007 law known as REACH (Registration, Evaluation, Authorization and Restriction of Chemicals), the agency requires companies that produce or import at least 1 ton of a chemical per year to submit detailed safety information on that substance. Those ECHA submissions are

chunks of text, which are difficult to analyze on a large scale.

To solve that problem, the researchers used software to extract text from 816,000 ECHA documents, which refer to some 9800 chemicals. Next, they analyzed the safety findings and organized compounds by their effects. They found that about 20% were labeled as skin sensitizers, for instance, and 17% irritate eyes. Finally, they created a visualization that displays the toxicological properties of the chemicals, and clusters them by their struc-

ture and can interact with each other."

But the approach has limits. In some cases, what really matters is not only a chemical's structure, Bahadori says, but also how organisms are exposed and respond to it. "The idea that we're going to do this based on similarity of structure is overly simplistic," warns biomedical researcher Andre Nel, who studies the safety of nanomaterials at the University of California, Los Angeles. Still, Nel says "starting with pockets of knowledge," such as the eye

irritation data, "is a useful, practical way to begin."

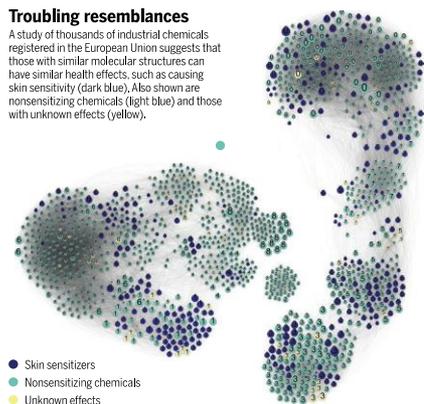
Legal obstacles could also hamper use of the screening method. Although ECHA's safety reports are public, the companies that registered the chemicals own the data in the reports. That could complicate Hartung's plans to share his database with other researchers and create a spinoff company that would help clients screen new chemicals. He is currently negotiating with the agency over the use of the data; an ECHA spokesperson says "we are keen to see data on chemicals being made use of to enhance their safe use, promote innovation, and avoid unnecessary testing on animals ... [but] we need to be sure that the appropriate rights have been respected."

In the meantime, Hartung says that "the fact that we got this public data is fantastic." But he says ECHA and other regulators could do more to make it usable by requiring companies to submit standardized, organized data instead of just text. His team is also meeting with experts in the United States and Europe to share practical read-across guidance, which he hopes will help the method catch on more quickly among regulators, academics, and industrial users.

Nel says that's a "sensible and laudatory goal" but predicts it will take years for such methods to be accepted internationally. Until then, Bahadori says, "we can make the chemistry of the future much smarter by making data available." ■

Troubling resemblances

A study of thousands of industrial chemicals registered in the European Union suggests that those with similar molecular structures can have similar health effects, such as causing skin sensitivity (dark blue). Also shown are nonsensitizing chemicals (light blue) and those with unknown effects (yellow).

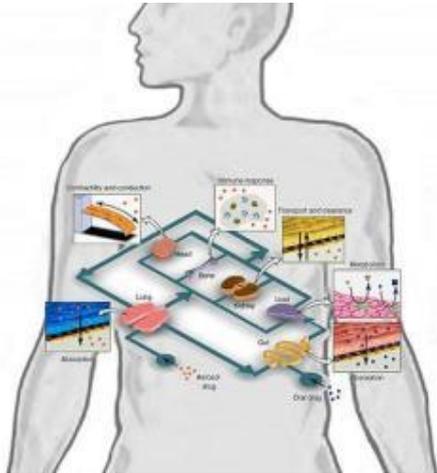


● Skin sensitizers
● Nonsensitizing chemicals
● Unknown effects

structural similarities (see image, above). It shows, for example, that some clusters are "full of problematic substances," Hartung says.

Such maps could ultimately help researchers evaluate the risks posed by new chemicals without testing them in vitro or in vivo, Hartung says, saving time, money, and possibly the lives of millions of lab animals. That hope has driven other predictive efforts, but they have generally lacked the extensive data underlying the new effort. Bahadori applauds the team for "unearthing and making the [ECHA] data accessible." And she predicts that "these tools will really show their promise ... as more decision-making bodies invest in generating data that are compara-

- Inter national, cross-industry and multi-stakeholder steering group
- One workshop and two symposia; 2017 regulator summit
- Good Read-Across Practice and guidance on biological support data
- Application to Green Toxicology (frontloading of toxicology)
- Automated tool development
- Collaboration with UL making tool and services available
- Expansion to other data-bases including pharma
- Additional uses e.g. drug contaminants



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

**21st century
science starts with
21st century cell
culture**



**Stem cells &
Organo-typic
culture & High-
content**



Promotion of organotypic cultures and Integrated testing Strategies



t4 Workshop Report*

State-of-the-Art of 3D Cultures (Organs-on-a-Chip) in Safety Testing and Pathophysiology

Natalie Alépée¹, Anthony Bahinski², Mardas Daneshian³, Bart De Wever⁴, Ellen Fritsche⁵, Alan Goldberg⁶, Jan Hansmann⁷, Thomas Hartung^{3,6}, John Haycock⁸, Helena T. Hogberg⁶, Lisa Hoelting⁹, Jens M. Kelm¹⁰, Suzanne Kadereit⁹, Emily McVey¹¹, Robert Landsiedel¹², Marcel Leist^{3,9}, Marc Lübberstedt¹³, Fozia Noor¹⁴, Christian Pellevoisin¹, Dirk Petersohn¹⁵, Uwe Pfannenbecker¹⁶, Kerstin Reisinger¹⁵, Tzutzy Ramirez¹², Barbara Rothen-Rutishauser¹⁷, Monika Schäfer-Korting¹⁸, Katrin Zeilinger¹³ and Marie-Gabriele Zurich^{19,20}



t4 Workshop Report*

Biology-Inspired Microphysiological System Approaches to Solve the Prediction Dilemma of Substance Testing

Uwe Marx¹, Tommy B. Andersson^{2,3}, Anthony Bahinski⁴, Mario Beilmann⁵, Sonja Beken⁶, Flemming R. Cassee^{7,8}, Murat Cirit⁹, Mardas Daneshian¹⁰, Susan Fitzpatrick¹¹, Olivier Frey¹², Claudia Gaertner¹³, Christoph Giese¹⁴, Linda Griffith⁹, Thomas Hartung^{10,13}, Minne B. Heringa⁷, Julia Hoeng¹⁶, Wim H. de Jong⁷, Hajime Kojima¹⁷, Jochen Kuehl¹⁸, Marcel Leist¹⁰, Andreas Luch¹⁹, Ilka Maschmeyer¹, Dmitry Sakharov²⁰, Adrienne J. A. M. Sips⁷, Thomas Steger-Hartmann²¹, Danilo A. Tagle²², Alexander Tonevitsky²³, Tewes Tralau¹⁶, Sergej Tsyb²⁴, Anja van de Stolpe²⁵, Rob Vandebriel⁷, Paul Vulto²⁶, Jufeng Wang²⁷, Joachim Wiest²⁸, Marleen Rodenburg⁷ and Adrian Roth²⁹

Concept articles, workshops and symposia Transatlantic Think Tank for Toxicology

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

Thomas Hartung^{1,2}, Tom Luechtefeld¹, Alexandra Maertens¹, and Andre Kleensang¹

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

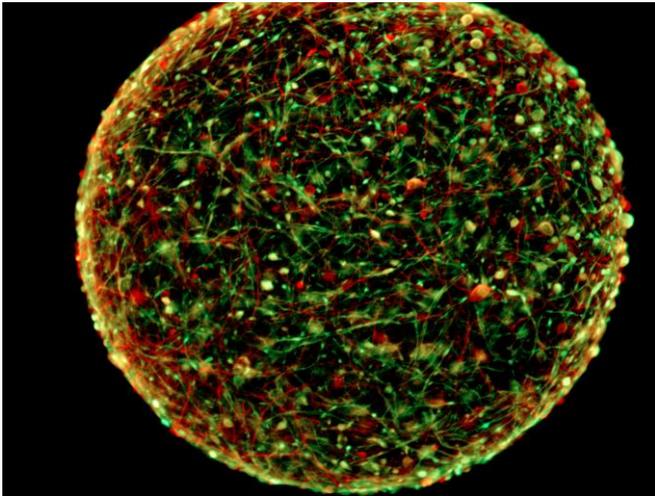


t4 Workshop Report*

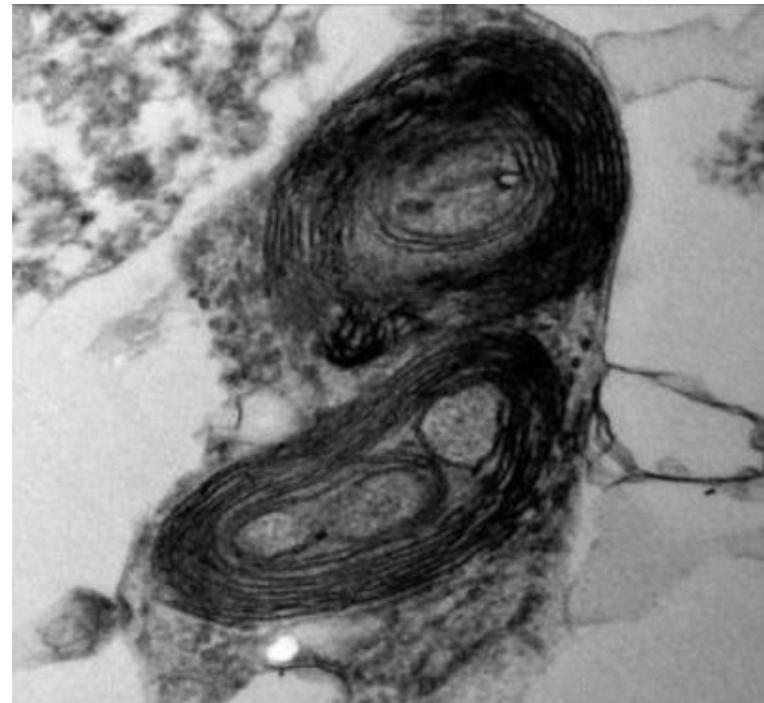
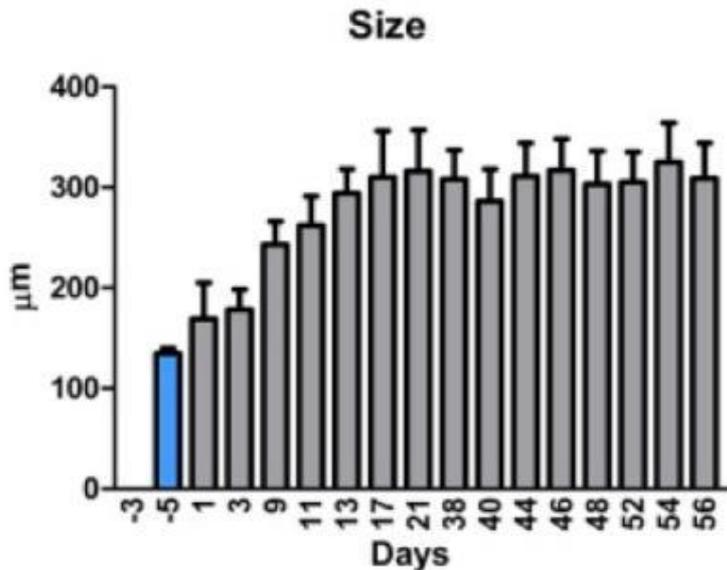
Integrated Testing Strategies (ITS) for Safety Assessment

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 Luechtefeld²⁰, Monica Locatelli²¹, Annette Mehling¹⁸, Andreas Natsch²², Jonathan W. Pitchford²³, Donald Prater²⁴, Pilar Prieto²⁵, Andreas Schepky²⁶, Gerrit Schüürmann^{27,28}, Lena Smirnova²⁹, Colleen Toole²⁹, Erwin van Vliet³⁰, Dirk Weisensee¹⁰ and Thomas Hartung^{1,20}

Our mini-brain project



- Stable size, no necrosis
- Stable composition (10% oligodendrocytes, 20% astrocytes, 5+ types of neurons)
- 40% myelinated axons
- Electrophysiologically active



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Charakter prägen

Trisomie 21

Ein Medikament gegen
das Downsyndrom?

Fossile Gehirne

Was machte den Mensch
zum Menschen?



**Human Cell-Based Tech Could Replace Lab
Animals**

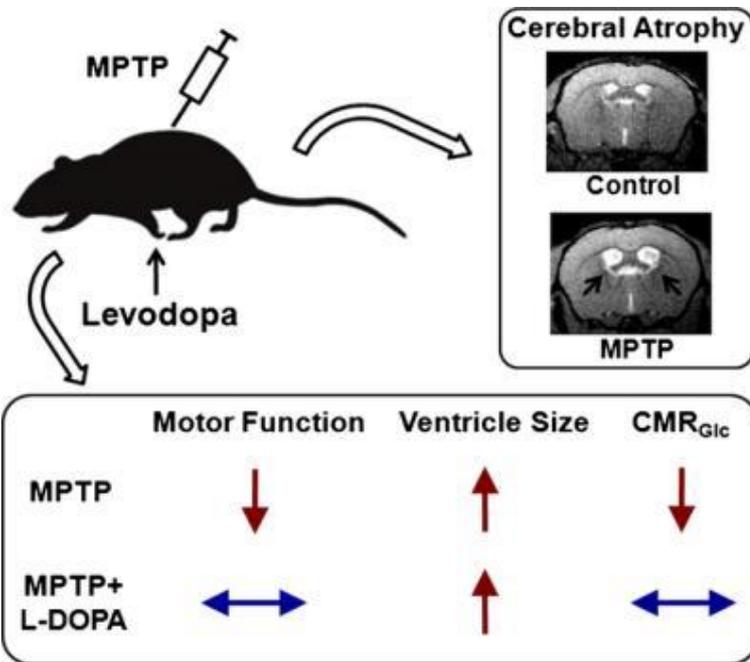
**Announced at AAAS 12
Feb 2016**

80+ articles

**Huge expectations
Some quote me
correctly:**

**But, right now, he
cautions, "I'm selling
hopes," as the mini-
brains are brand new
and haven't proven
useful yet.**

Meperidine addiction led to Parkinson via MPTP contamination



Standard animal model

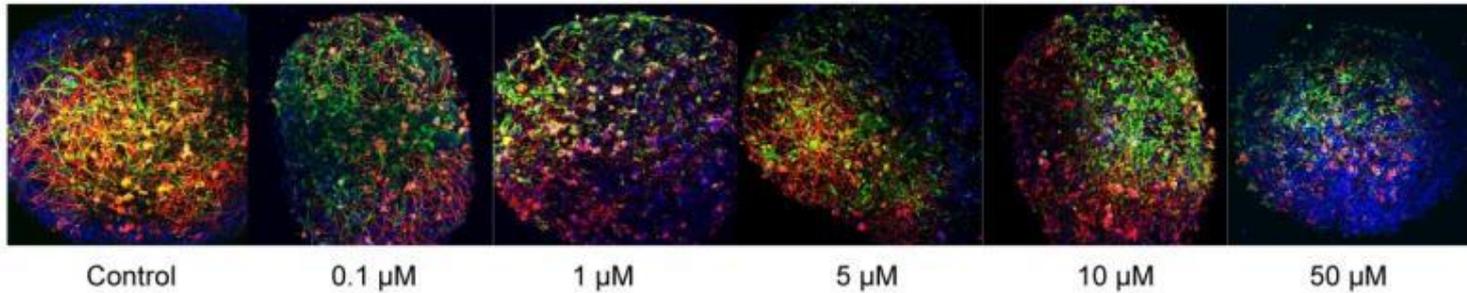
Similar: pesticide Rotenone



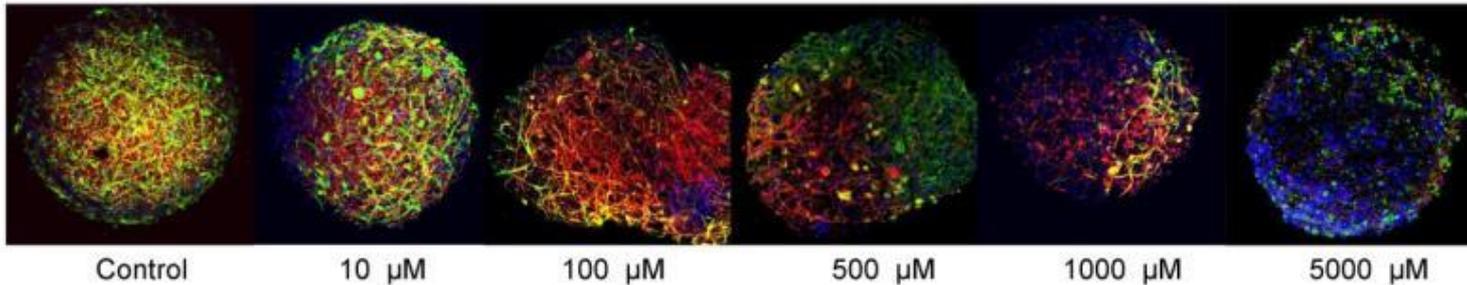
Parkinson model

MPTP and Rotenone selectively kill dopaminergic neurons

Rotenone



MPP+



Food for Thought ...

Developmental Neurotoxicity – Challenges in the 21st Century and *In Vitro* Opportunities

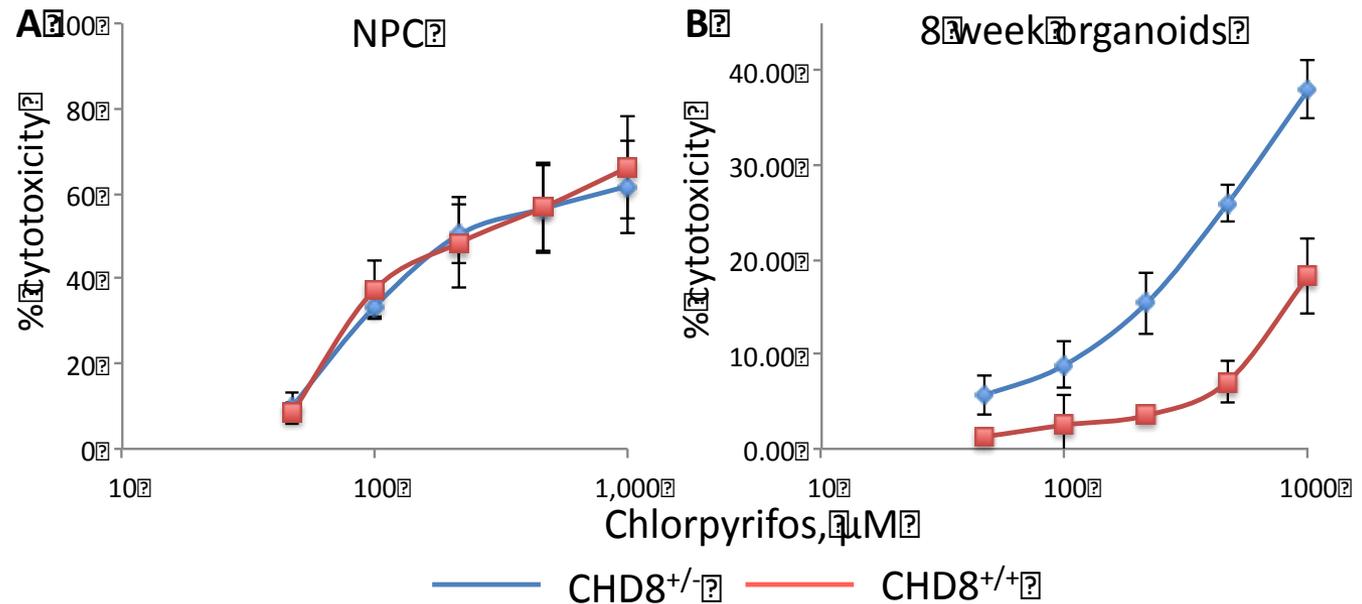
Lena Smirnova¹, Helena T. Hogberg¹, Marcel Leist², and Thomas Hartung^{1,2}

ALTEX 2014, 31:129-156

Diseases like autism cannot be explained by genetics or exposure alone

Disposition to DNToxicants?

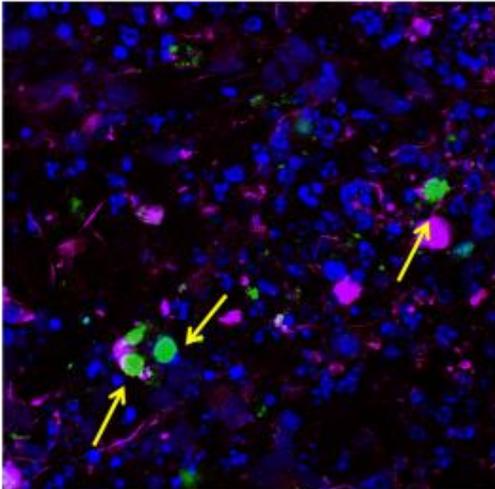
Test on a genetic background that allows DNT



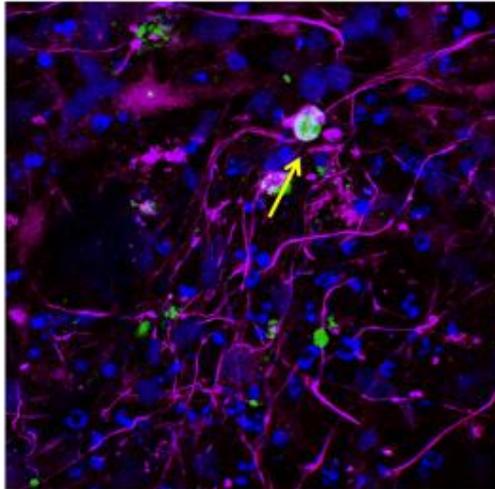
Synergy of CHD8 and chlorpyrifos, i.e. two ASD risk factors

MAD – 7 WKS: 2 WKS POST INFECTION

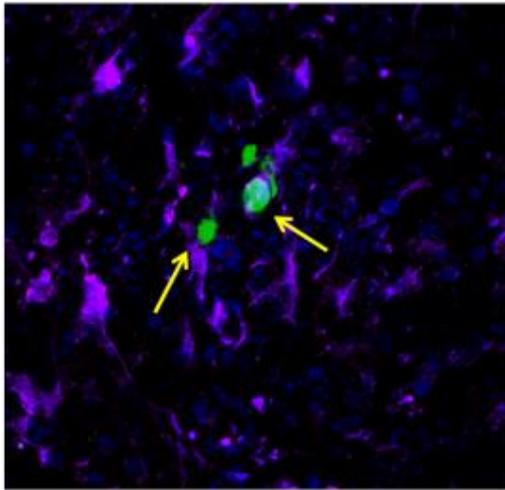
PAB/GFAP



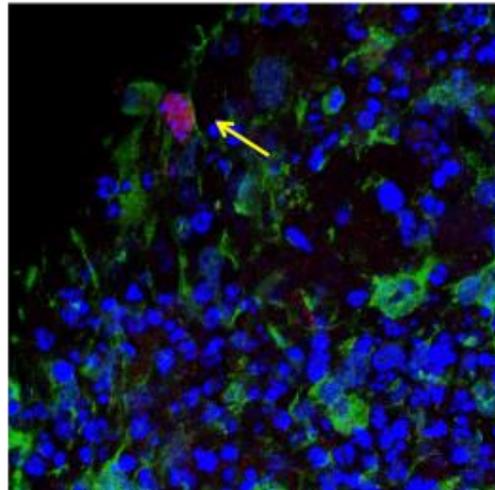
PAB/GFAP



PAB/NOGOA



Olig1/SV40



Use for viral
infection

Examples:

JC virus

Zika

HIV

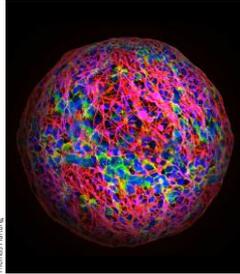
<http://www.organome.co>

NEWS

New company aims to broaden researchers' access to organoids

Ever since organoids were first developed a decade ago, researchers with the know-how to create these miniature, organ-like structures have also been the main people using them. That may soon change: as scientists in the US wait for government institutions to create organoid banks for research, at least one company is preparing to commercialize organoids for study.

At the American Association for the Advancement of Science's annual meeting in February, Thomas Hartung introduced Organome, a Baltimore-based company formed to mass-produce brain organoids for research. Hartung, director of Johns Hopkins University's Center for Alternatives to Animal Testing, also announced the development of a new brain-organoid model to be sold through the company. Not



Sphere for study: A brain organoid from Hartung's lab.

out to guide other scientists through the process of commercializing organoids.

"We're constantly frustrated by the lack of access to things generated in academic labs," says Elizabeth Iorns, co-founder of ScienceExchange, a website that enables scientists who lack equipment or expertise to outsource projects to labs with the proper materials.

Iorns also thinks that Hartung's ability to deliver consistent products—given that his organoids are derived from induced pluripotent stem cells, which are difficult to standardize—is key: "What Thomas is doing is really great and will provide standardized materials for a lot of people."

"I haven't come across anything like this before," says James Wells, director of the Pluripotent Stem Cell Center at the

Goals:

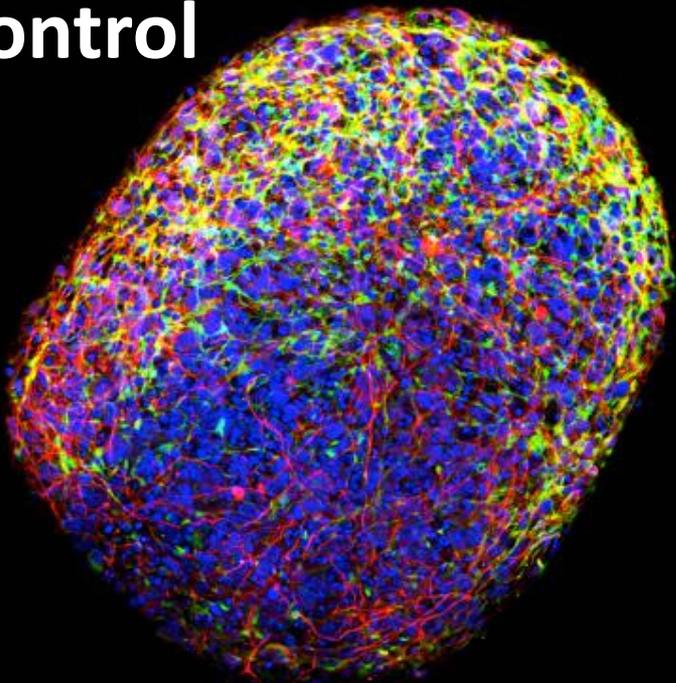
- **Not Brainome but Organome**
- **Get Organoids out of academia to practical use**
- **Help standardization, QA and validation**
- **Make visible**

Hartung says. "There'll be lots of bottles of champagne when we figure this out."



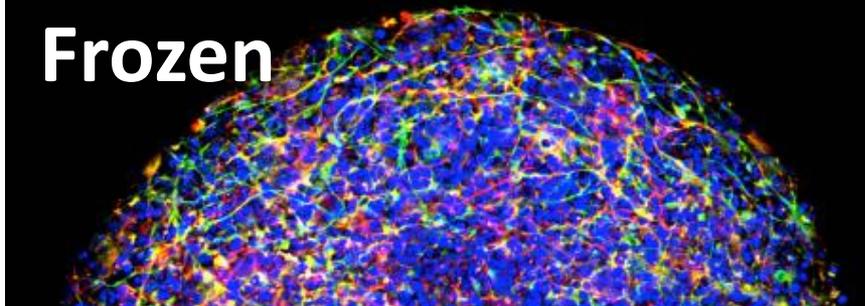
ORGANOME_{pr} 2016

Control

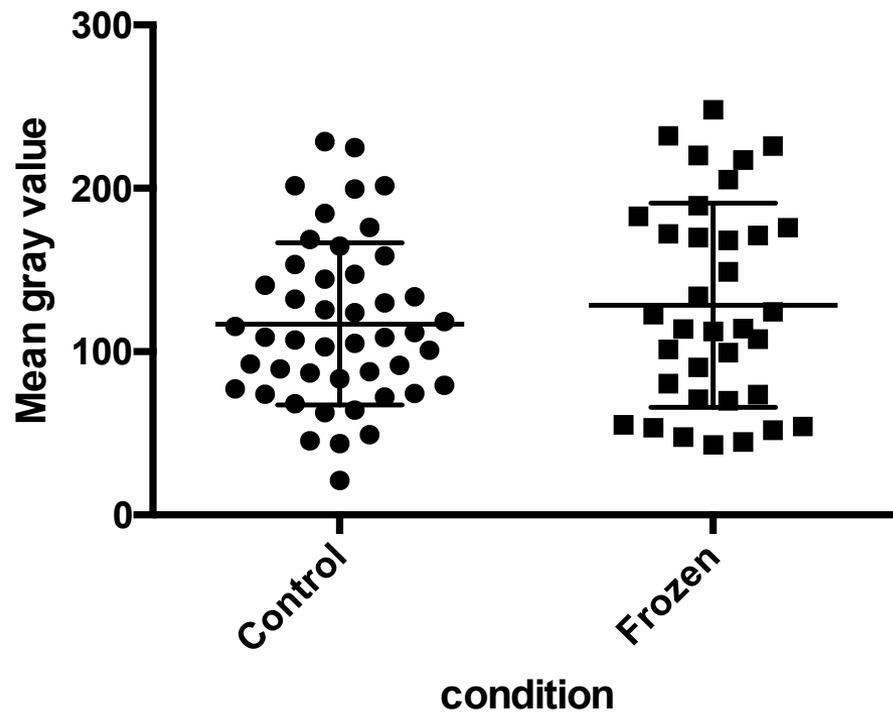


NF/TH/DAPI

Frozen



Mitotracker



Cryopreservation of mini-brains - Neurite outgrowth -

Control

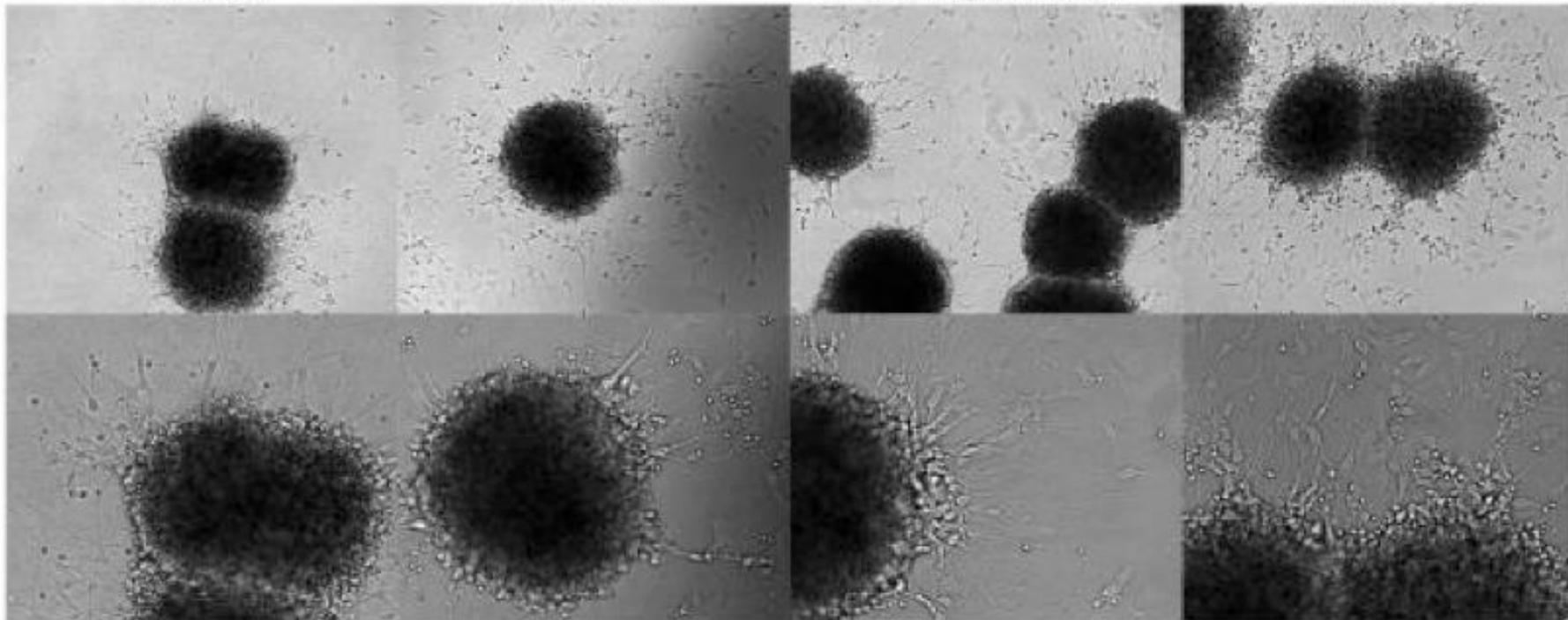
Cryostor

Heating packs

Ice

10x

20x

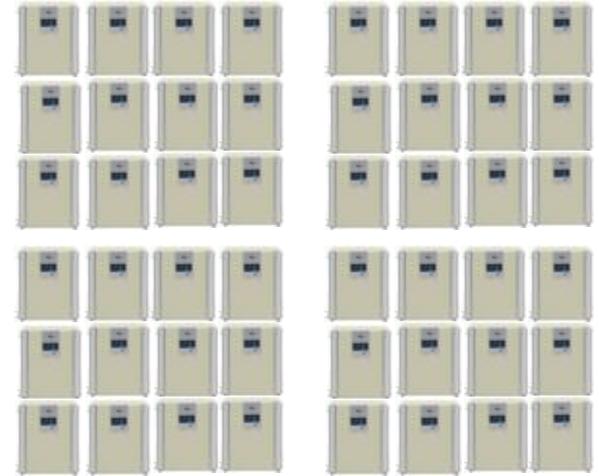


The advantage of “brain on the rocks”



**Academic model
in cell incubator**

**Several cell lines
continuous production**

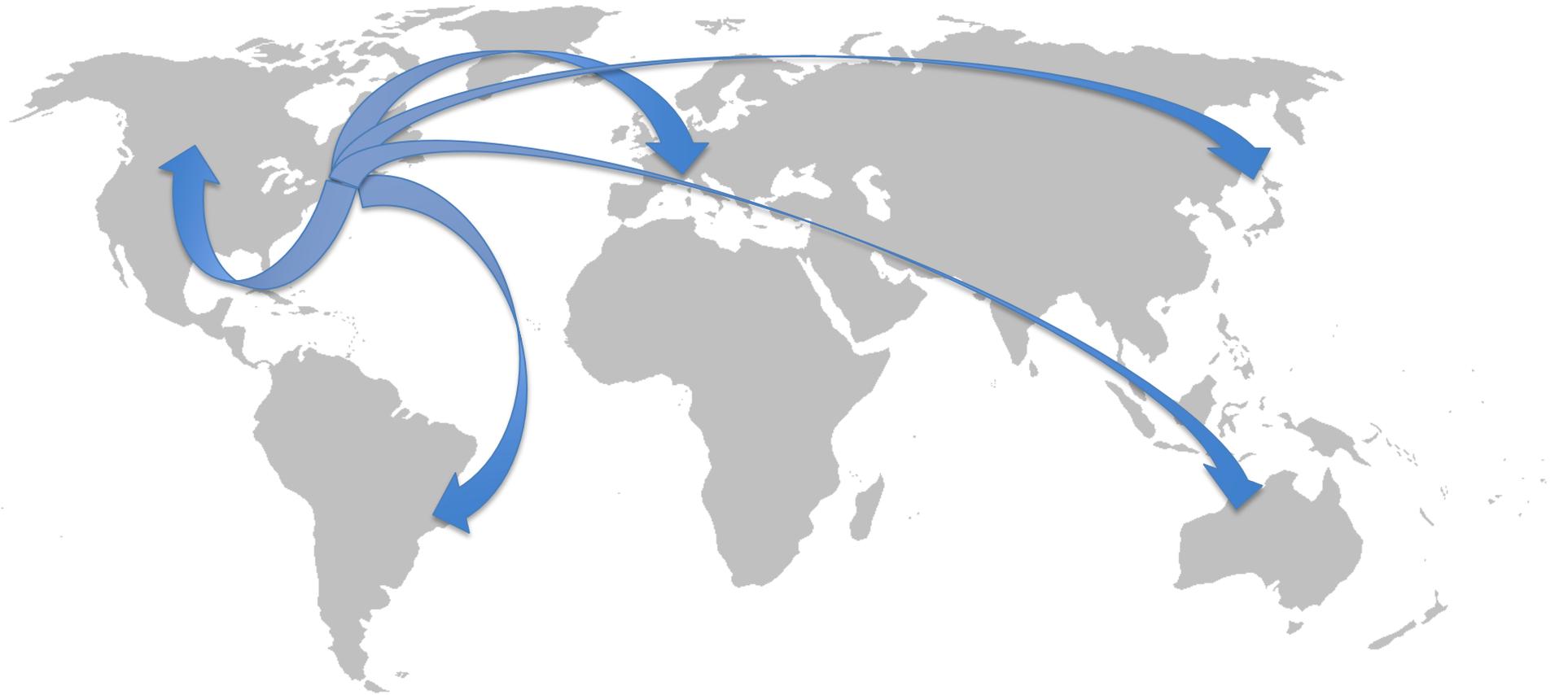


**Weekly
production 3
month model**

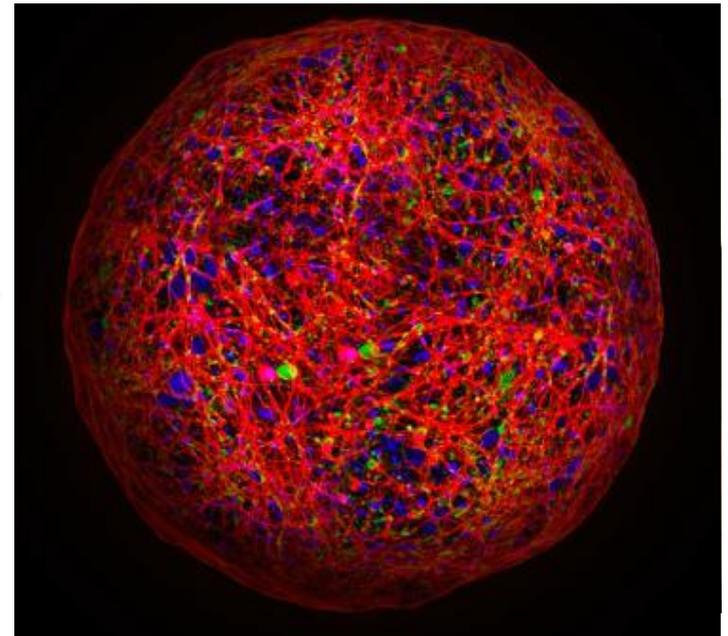
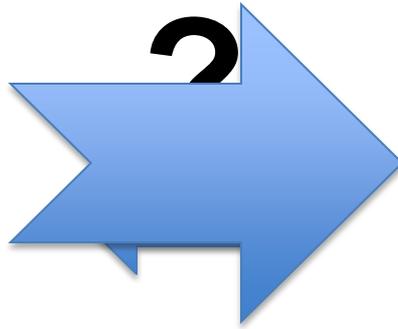


Stockpiling of frozen mini-brains

Get frozen mini-brains where they are needed



Are our cell cultures good enough?



I cannot say whether things will get better if we change; what I can say is they must change if they are to get better.



**Georg Christoph Lichtenberg
(1742 - 1799)**