



JOHNS HOPKINS  
BLOOMBERG SCHOOL  
of PUBLIC HEALTH



Slides available



Thomas Hartung & team

Toxicology for the  
21<sup>st</sup> Century 2.0

Some pictures removed for copyright reasons

BfR  
2  
GO



Hunting for risks

Our BfR2GO cover story on 20 years of risk assessment

BfR Science Magazine BfR2GO  
Issue 01/2022



# Thanking our sponsors

## Current



Space for You!



## Recent



## Philanthropic



...and individuals





 **frontiers**  
in Artificial Intelligence

**Field Chief Editor**

 **frontiers**  
in Big Data

**Chief Editor**

Medicine & Public Health

AstraZeneca 

MPS



**ATCC**



**AxoSim**  
Human Data, Faster.  
VP, shareholder

*in*sphero

Apellis



Pyrogen



A.I.



 **Consultant**

**Green Chemistry  
Advisory Panel**

**ToxTRACK**

**Consultant, shareholder**

**In preparation: Insilica LLC**





# Der lange Weg zur validierten Ersatzmethode

Thomas Hartung\* und Horst Spielmann\*\*



**1995: “The sophisticated way to a validated alternative method” .... Set the theme for my next 25 years!**

**1992-1993 paid as post-doc by a grant to me from ZEBET**

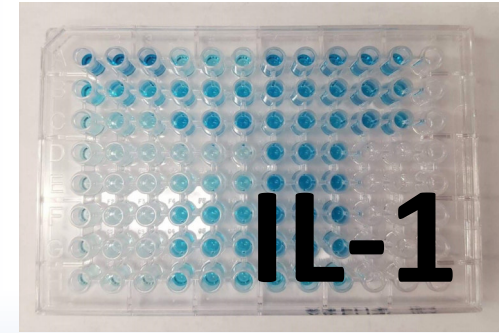
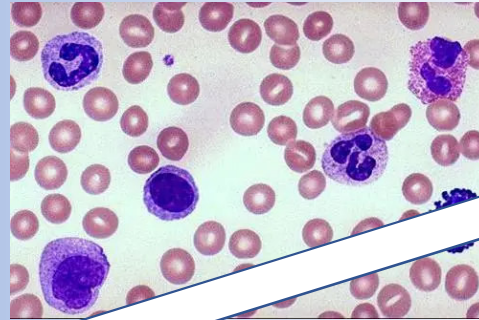
**2011 recruited BfR post-doc Lena Smirnova to CAAT and into my personal life.**





# Human(e) Pyrogen Tests

Rabbit pyrogen test



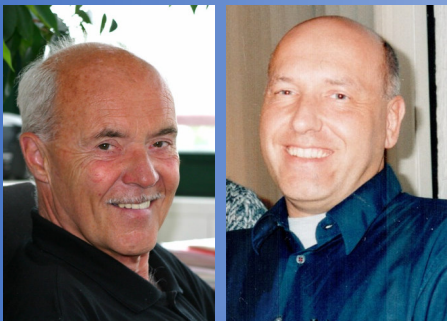
Fever

Whole Blood

Fever signals  
of the body

+

Sample



Albrecht Wendel & myself

## Entwicklung und Evaluierung eines Pyrogentests mit menschlichem Blut

Thomas Hartung, Stefan Fennrich, Matthias Fischer\*, Thomas Montag-Lessing\* und Albrecht Wendel  
Universität Konstanz, Biochemische Pharmakologie, D-Konstanz, \*Paul-Ehrlich-Institut, D-Langen

*Dieses Projekt wurde gefördert durch ZEBET, D-Berlin,  
BMBF, D-Bonn und set, D-Mainz.*

ALTEX 1998, 15:9-10.

Research paper

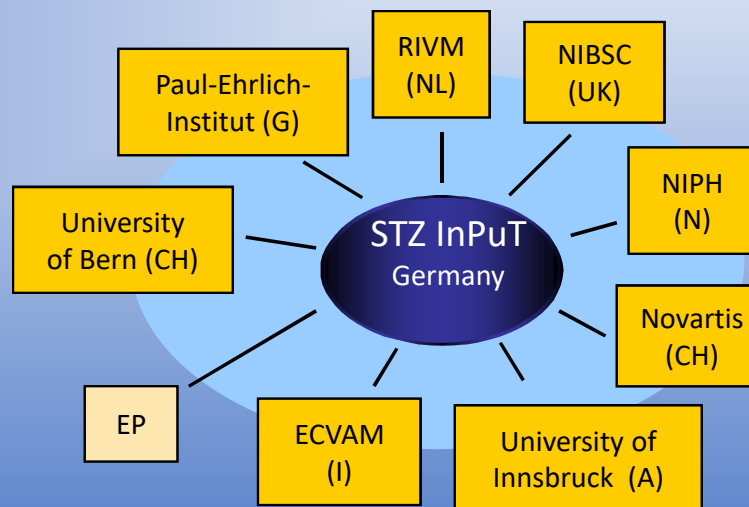
## International validation of novel pyrogen tests based on human monocytoid cells

Sebastian Hoffmann<sup>a,h,1</sup>, Anja Peterbauer<sup>e,1,2</sup>, Stefanie Schindler<sup>a,1</sup>, Stefan Fennrich<sup>a</sup>,  
Stephen Poole<sup>b</sup>, Yogesh Mistry<sup>b</sup>, Thomas Montag-Lessing<sup>c</sup>, Ingo Spreitzer<sup>c</sup>,  
Bettina Löschner<sup>c</sup>, Mirjam van Aalderen<sup>d</sup>, Rogier Bos<sup>d</sup>, Martin Gommer<sup>d</sup>,  
Ria Nibbeling<sup>d</sup>, Gabriele Werner-Felmayer<sup>e</sup>, Petra Loitzl<sup>e</sup>, Thomas Jungi<sup>f</sup>,  
Marija Bricic<sup>f</sup>, Peter Brügger<sup>g</sup>, Esther Frey<sup>g</sup>, Gerard Bowe<sup>h</sup>, Juan Casado<sup>h</sup>,  
Sandra Coecke<sup>h</sup>, Jan de Lange<sup>h</sup>, Bente Mogster<sup>i</sup>, Lisbeth M. Næss<sup>i</sup>,  
Ingeborg S. Aaberge<sup>i</sup>, Albrecht Wendel<sup>a</sup>, Thomas Hartung<sup>a,h,\*</sup>



## Collaborative study

- 2000-2003
- 10 partners
- 6 cell tests
- About \$3 million
- Validated 2006



**4 out of 6 tests  
validated:  
(Cryo-) Whole blood,  
white blood cells,  
(a leukemia cell line)**

# Pyrogen testing finally vanishing

2005: ~160,000 rabbits

2008: ~170,000 rabbits

2015: 46,553

2016: 39,434

2017: 35,172

2018: 30,453

2019: 30,912

**30 years to full  
implementation**



**News 2021: Europe will  
phase out rabbits within 5a!**



# Regulatory science is the art of decelerating progress

Illustration deceleration



**Or repeated low dose...**

Implementing 10 years earlier

~ 1.5 million rabbits saved

~ 100 million € saved

Illustration deceleration trauma

Man riding turtle

Desperate man in front of  
House with door on second floor

**Europe**



**Thanks to REACH, the door for  
New Approach Methods is wide  
open....**

**...but thanks to ECHA, the door is  
difficult to reach**

**Still struggling to implement 20+  
years old methods**

~50% of Americans and  
~60% of Europeans  
object to animal testing

**Pressure is mounting**

Illustration Public Opinion

Politician interviewed by journalists

2002 EU cosmetics ban

2006 Goal of EU REACH

2016 Goal for US TSCA

2019 Deadline 2035 by US EPA

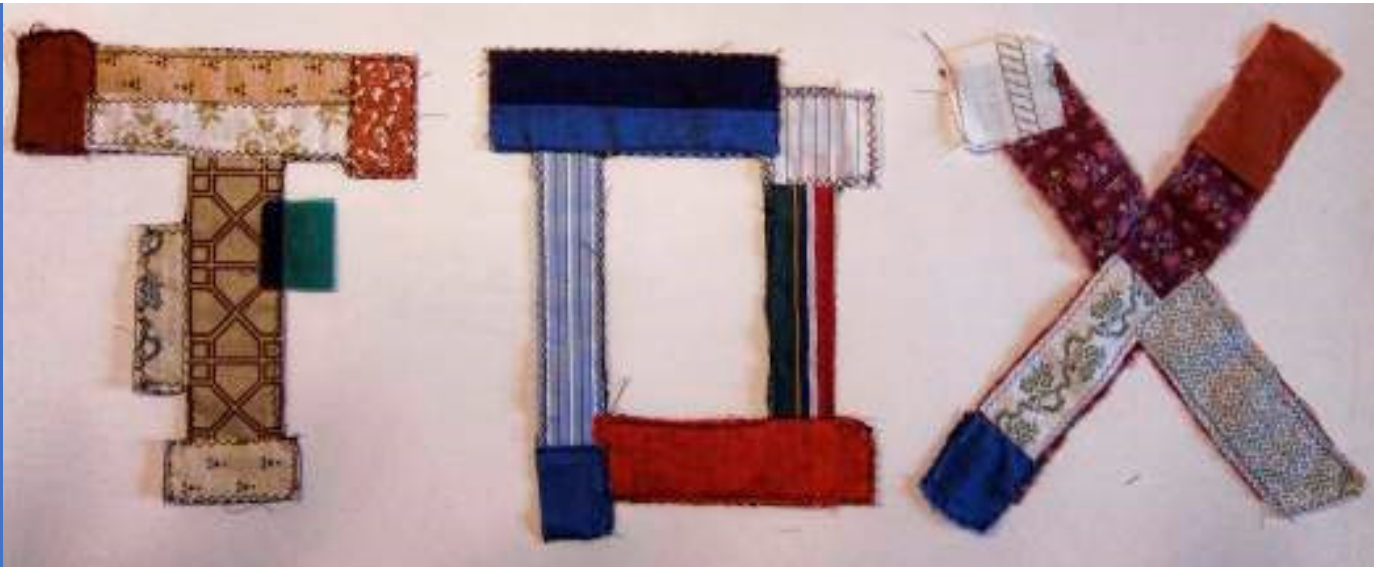
2021 Deadline 2027 by EFSA



# Toxicology is big science

Big science  
symbol

- Only a small percentage of disease is genetic, the rest is bad luck and EXPOSURE
- We actually prevent disease (unfortunately nobody knows how much)
- Almost all products would need risk assessment
- Toxicology pioneered quality (GLP) and validation (incl. relevance)
- Tox was the first preclinical science to adapt Evidence-based approaches



## *The evolution of toxicology: patchwork*

- Every scandal gives one patch.
- Many patches are 50-80 years old.
- No way to remove a patch.
- Every patch is of its own appearance and workmanship.

*No revolution  
in quite a while*

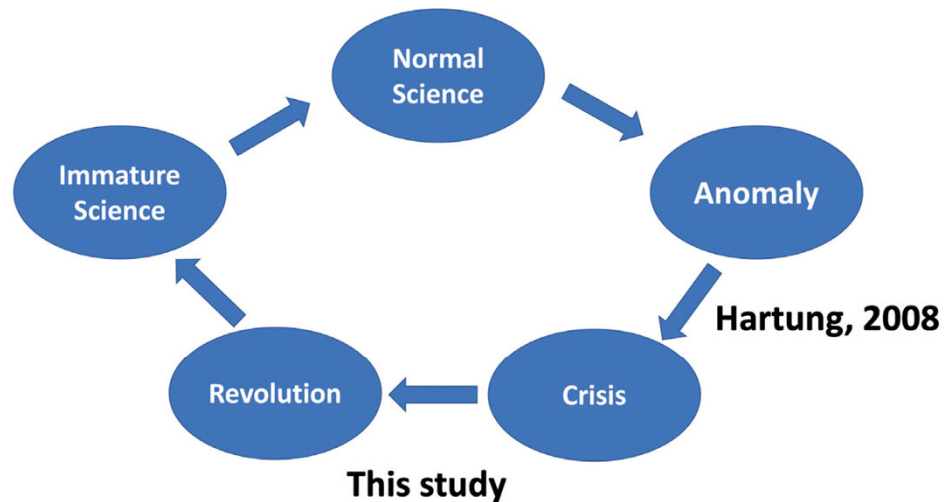


Fig. 1: Kuhn's Scientific Revolution Cycle (adapted from Kerry et al., 2008) and where the authors saw the state of regulatory toxicology in 2008 and now in 2021

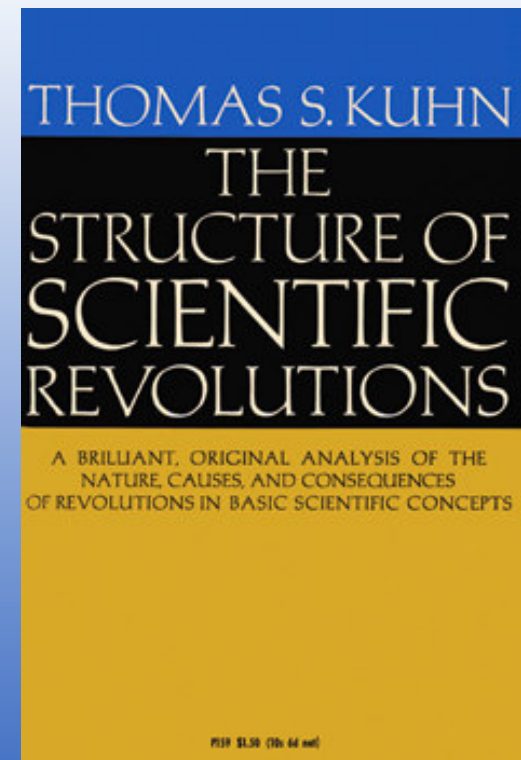
It is time for a scientific revolution in toxicology!

Food for Thought ...

ALTEX 2021

## The State of the Scientific Revolution in Toxicology

Thomas Hartung<sup>1,2</sup> and Aristides M. Tsatsakis<sup>3</sup>





# Lack of throughput

Cartoon showing many untested chemicals

**350,000 chemicals  
registered in 19 countries**

Copying and redistribution of the article or any adaptations for noncommercial purposes.

**ENVIRONMENTAL**  
Science & Technology

pubs.acs.org/est

ACS EDITORS' CHOICE

Policy Analysis

**Toward a Global Understanding of Chemical Pollution: A First Comprehensive Analysis of National and Regional Chemical Inventories**

Zhanyun Wang,\* Glen W. Walker, Derek C. G. Muir, and Kakuko Nagatani-Yoshida

**US: 1,000 chemical  
premarketing notifications  
per year**

**My Shopping List**

Skin Irritation	1.200 €
Skin Sensitization (LLNA)	4.700
Oral Acute Toxicity	4.500
Inhalation Acute Toxicity	3.900
Dermal Acute	1.500
Repeated Dose 28d	46.500
Repeated Dose 90d	106,000
Mutagenicity	62.500

**My Shopping List**

Carcinogenicity	700.000 €
Developmental Tox	63 – 112.000
ReproTox 1gen rat	77.700
ReproTox 1gen rabbit	126.000
ReproTox 2gen rat	328.000
ReproTox 2gen rabbit	481.000
Long-term fish	8.600

# Costs

**ALTEX 2018, 35:275-305**

Food for Thought ...

ALTEX 2018, 35:275-305

# Animal Testing and its Alternatives – the Most Important Omics is Economics

*Lucy Meigs<sup>1,2</sup>, Lena Smirnova<sup>2</sup>, Costanza Rovida<sup>3</sup>, Marcel Leist<sup>3</sup> and Thomas Hartung<sup>2,3</sup>*



**Symbols of economy**

**Economic considerations are underestimated**

**They promote and hinder implementation of NAM**



# Reproducibility

Six most frequent **toxicity tests**

Consuming **57%** of animals in tox

**350-750 chemicals with repeat tests**  
(n = 2,839, up to ~100 repeats)

**81% reproducible**

**69% reproducible for toxic chemicals**

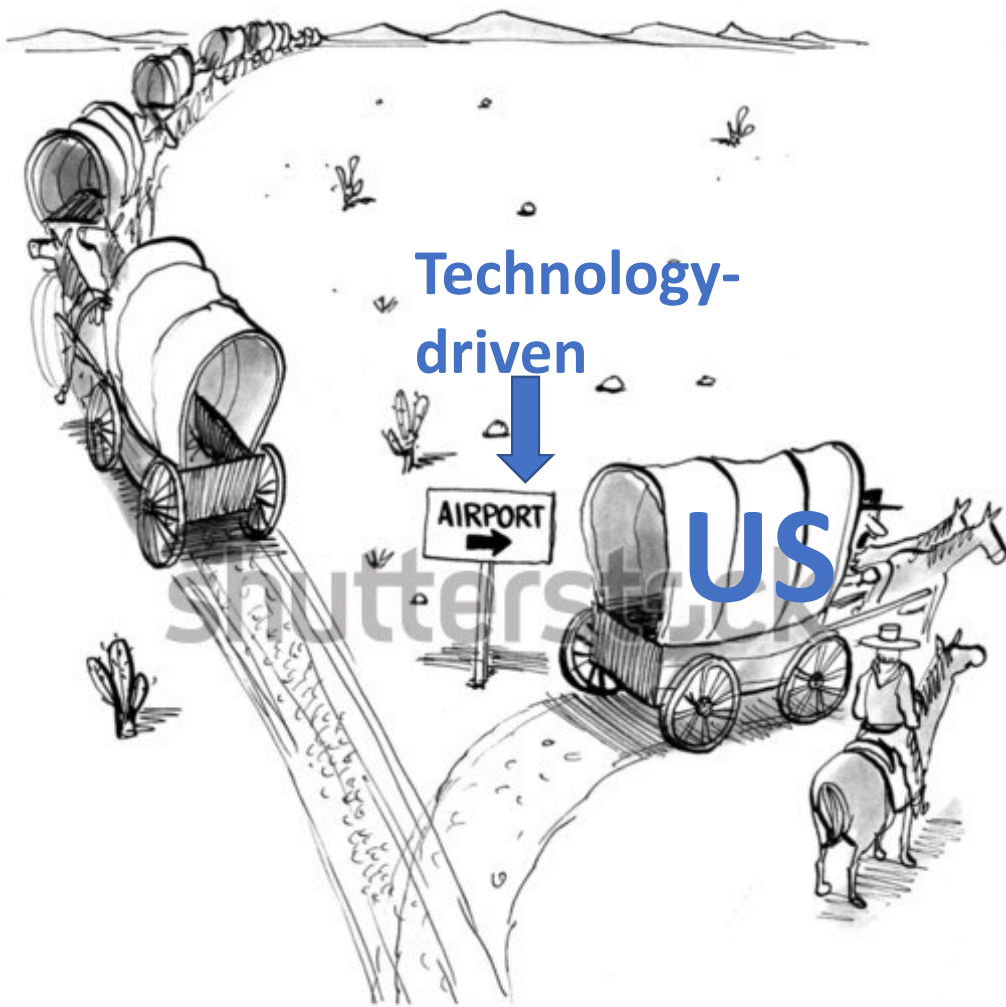
Mice and rat predict  
each other ~60%  
for systemic tox



Picture of rat



Luechtefeld et al., ToxSci 2018

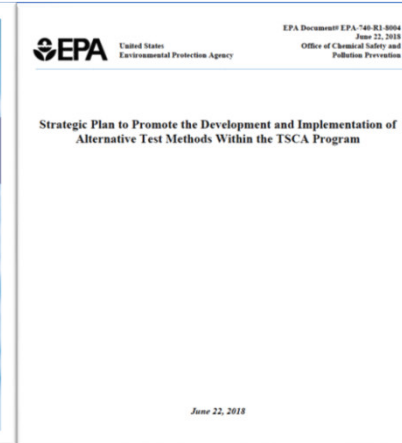
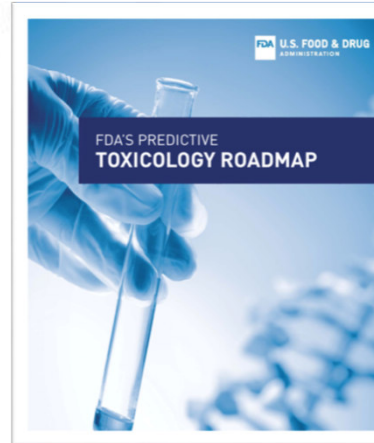


Technology-driven

US

"Uh, yeah, you go ahead. We'll catch up with the wagon train later."

www.shutterstock.com · 85849963



While Europe is slowly progressing....  
...a lot of good news comes from the US, who were trailing behind for so long

# Next generation - almost 16,000 active learners



Since 2018

Toxicology 21:  
Scientific Applications

Johns Hopkins University

**8500+ enrolled learners**



Since 2019

Evidence-based  
Toxicology

Johns Hopkins University

**7000+ enrolled learners**

COURSERA (Massive Open Online Courses platform)

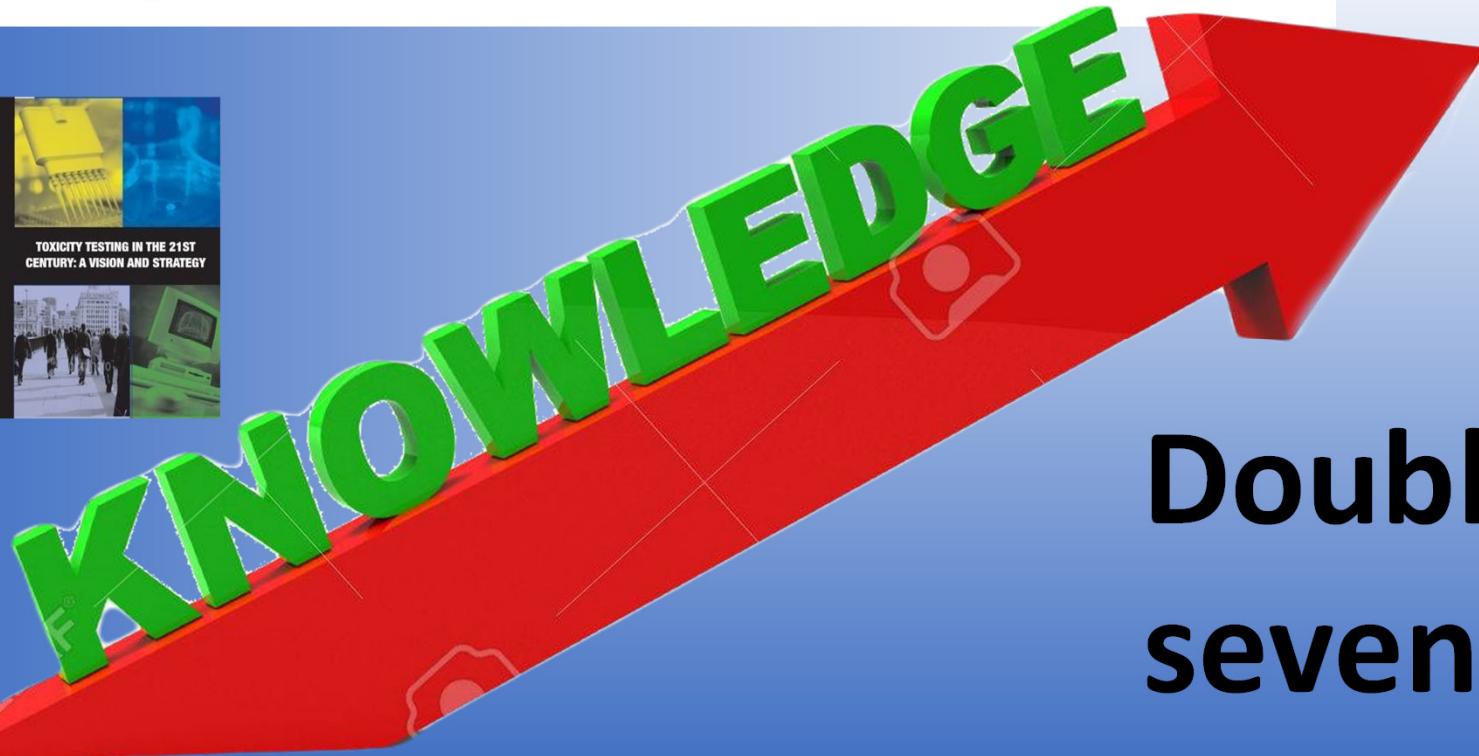
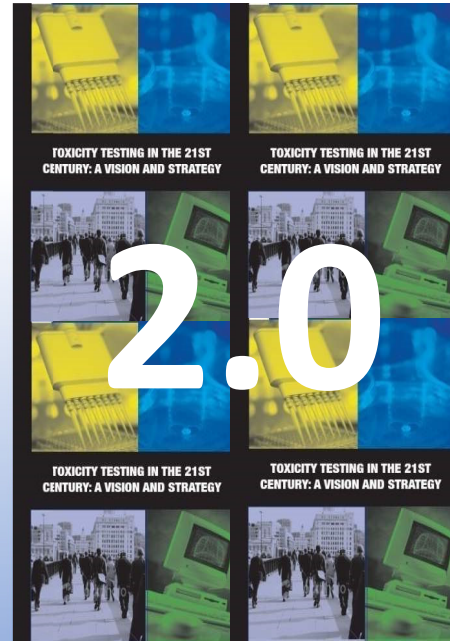
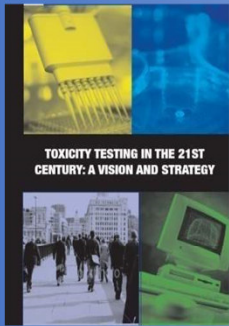
# Watershed moment 2007 NRC report



Toxicity testing in the 21st century: progress in the past decade  
and future perspectives

Arch Toxicol 2019

D. Krewski<sup>1,2,4</sup> · M. E. Andersen<sup>3</sup> · M. G. Tyshenko<sup>2,4</sup> · K. Krishnan<sup>2,5</sup> · T. Hartung<sup>6,13</sup> · K. Boekelheide<sup>7</sup> ·  
J. F. Wambaugh<sup>8</sup> · D. Jones<sup>9</sup> · M. Whelan<sup>10</sup> · R. Thomas<sup>8</sup> · C. Yauk<sup>11</sup> · T. Barton-Maclaren<sup>11</sup> · I. Cote<sup>12</sup>



## Doubling every seven years



# Scientific progress

Symbol Disruptive Technologies

Visualization of acceleration

# Acceleration



Future Directions  
Workshop: Advancing  
the Next Scientific  
Revolution in  
Toxicology

April 28-29, 2022

Thomas Hartung, Johns Hopkins University, University of Konstanz,  
and Georgetown University

Ana Navas-Acien, Columbia University  
Weihsueh Chiu, Texas A&M University

Prepared by:  
Kate Kramo, Virginia Tech Applied Research Corporation  
Matthew Peters, Virginia Tech Applied Research Corporation  
Shawn Silberberg, Office of the Under Secretary of Defense  
Research & Engineering, Basic Research Office

Future Directions Workshop series  
Workshop sponsored by the Basic Research Office, Office of  
the Under Secretary of Defense for Research & Engineering

**VT-ARC**  
Virginia Tech  
Applied Research Corporation

Releasable to the US Government Only and its Contractors | Unclassified

# Call for a Human Exposome Project, in press

## Future Directions Workshop: Advancing the Next Scientific Revolution in Toxicology

Office of the Under Secretary of Defense for Research and Engineering OUSD(R&E)

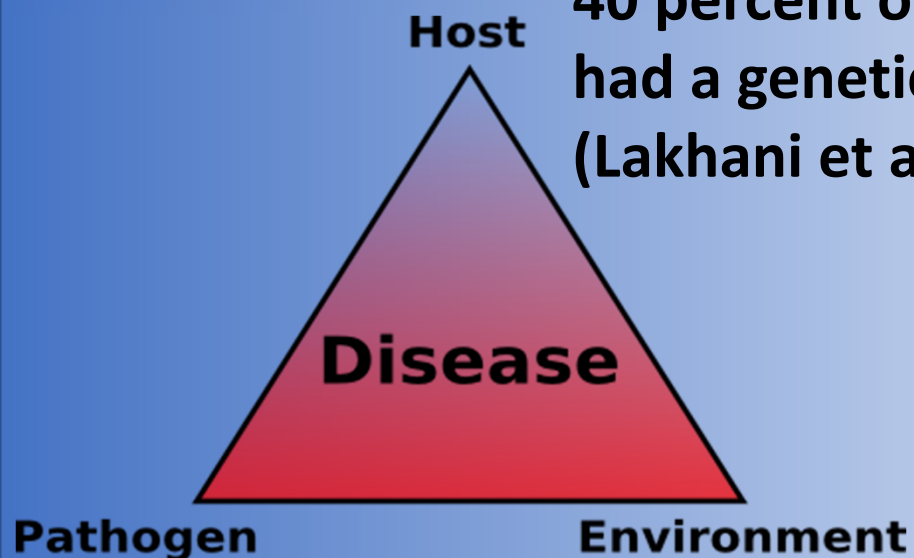
April 28–29, 2022

Arlington, VA

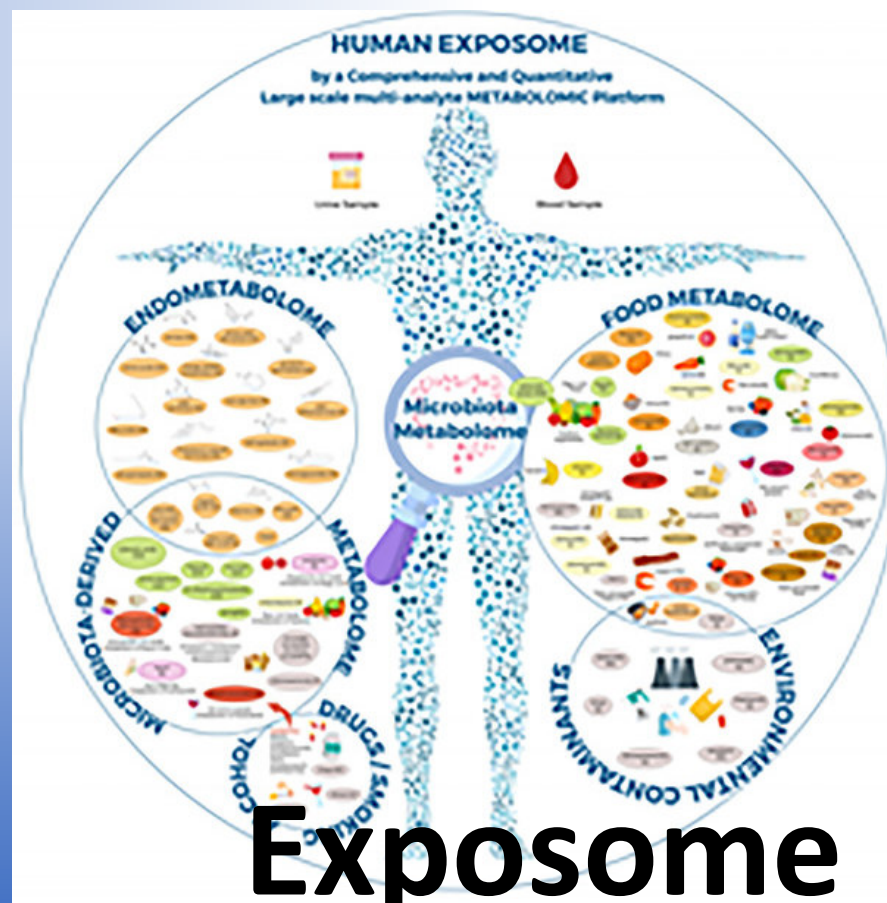
### Co-Chairs

**Ana Navas-Acien, Weihsueh A. Chiu &  
Thomas Hartung**

**~40 years of Human Genome:  
40 percent of 560 diseases  
had a genetic component  
(Lakhani et al., 2019)**



**70 to 90% of disease  
risks due to differences  
in environments  
(Rappaport and Smith,  
2010)**

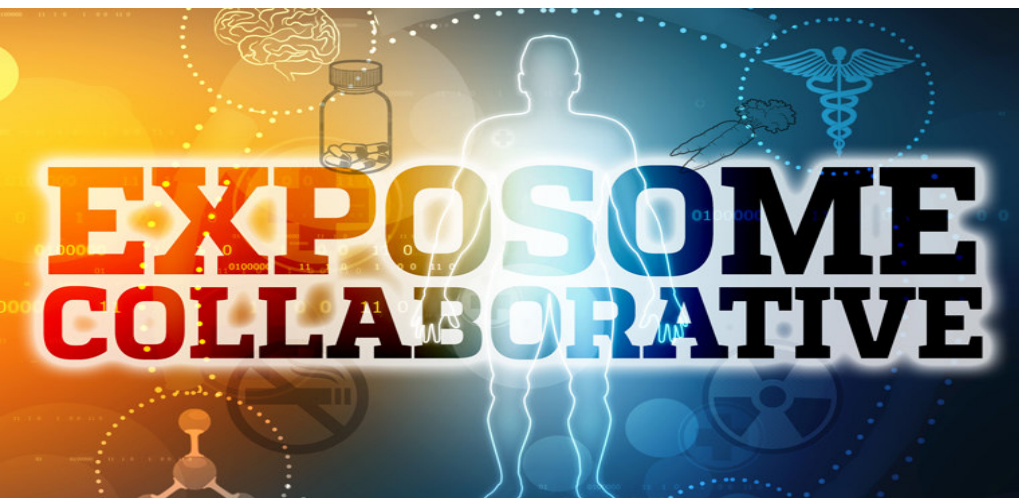


**What is emerging that can help us?**

**Exposure science** (high throughput and  
untargeted exposomics, remote sensing,  
citizen science ...)

**Technologies** (~omics, high-throughput, MPS, A.I.)

**Evidence Integration** (Evidence-based Tox, IATA, Green Tox  
Investigative Tox, Mechanistic Validation, Probabilistic  
Risk Assessment, Systems Toxicology, virtual  
experiments...)



Fenna Sillé



ALTEX 2020, 37, 3-23

*“Progress is impossible without change, and those who cannot change their minds cannot change anything.”*

George Bernard Shaw (1856-1950)

*“If you change the way you look at things, the things you look at change.”*

Wayne Dyer (1940-2015)

**Exposome  
& A.I.  
= E.I.  
(Exposome  
Intelligence)**

Food for Thought ...

## The Exposome – a New Approach for Risk Assessment

Fenna Sillé<sup>1</sup>, Spyros Karakitsios<sup>2</sup>, Andre Kleensang<sup>1</sup>, Kirsten Koehler<sup>1</sup>, Alexandra Maertens<sup>1</sup>, Gary W. Miller<sup>3</sup>, Carsten Prasse<sup>1</sup>, Lesliam Quiros-Alcala<sup>1</sup>, Gurumurthy Ramachandran<sup>1</sup>, Stephen M. Rappaport<sup>4</sup>, Ana M. Rule<sup>1</sup>, Denis Sarigiannis<sup>2,5</sup>, Lena Smirnova<sup>1</sup> and Thomas Hartung<sup>1,6</sup>



**What is emerging that can help us?**

**Exposure science** (high throughput and untargeted exposomics, remote sensing, citizen science ...)

**Technologies** (~omics, high-throughput, MPS, A.I.)

**Evidence Integration** (Evidence-based Tox, IATA, Green Tox Investigative Tox, Mechanistic Validation, Probabilistic Risk Assessment, Systems Toxicology, virtual experiments...)

# Progress in Computing

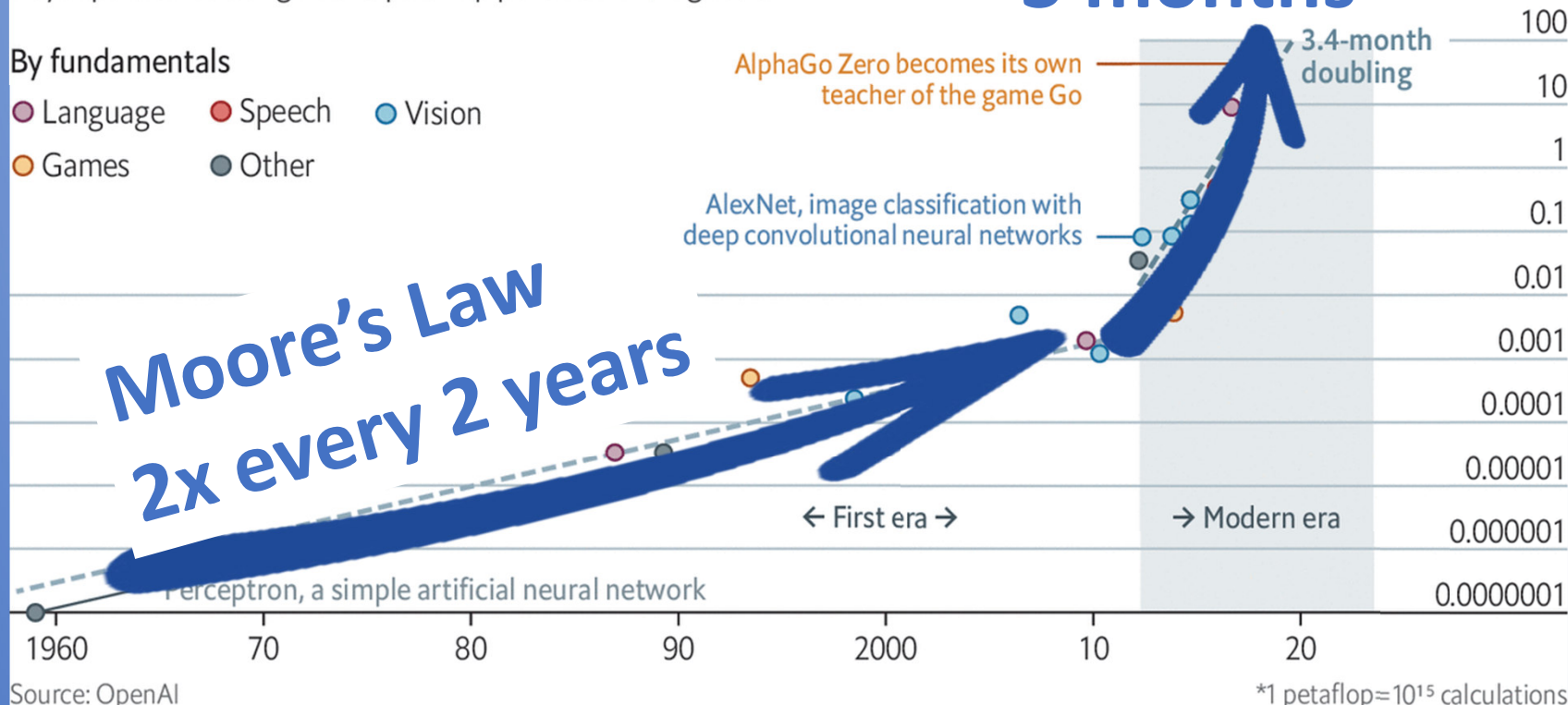
## Deep and steep

Computing power used in training AI systems

Days spent calculating at one petaflop per second\*, log scale

By fundamentals

- Language
- Speech
- Vision
- Games
- Other



Source: OpenAI  
The Economist

## AI - 2x every 3 months

100  
10  
1  
0.1  
0.01  
0.001  
0.0001  
0.00001  
0.000001  
0.0000001

**10<sup>9</sup>x**

# A.I. = Making big sense of

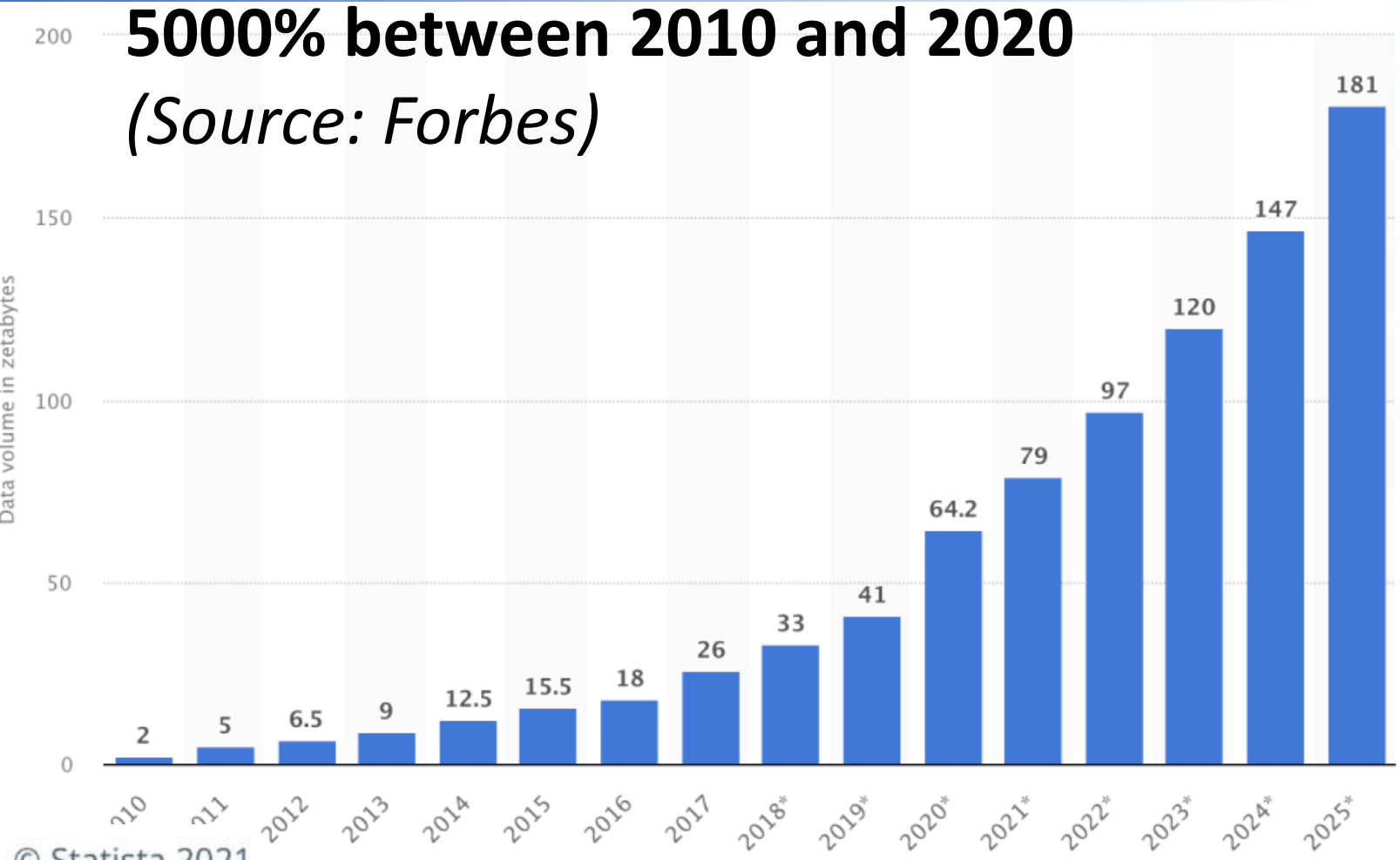


<https://theamericangenius.com/editorials/big-data-is-watching-you-some-will-panic-others-will-rejoice/>

# Data produced world-wide

**5000% between 2010 and 2020**

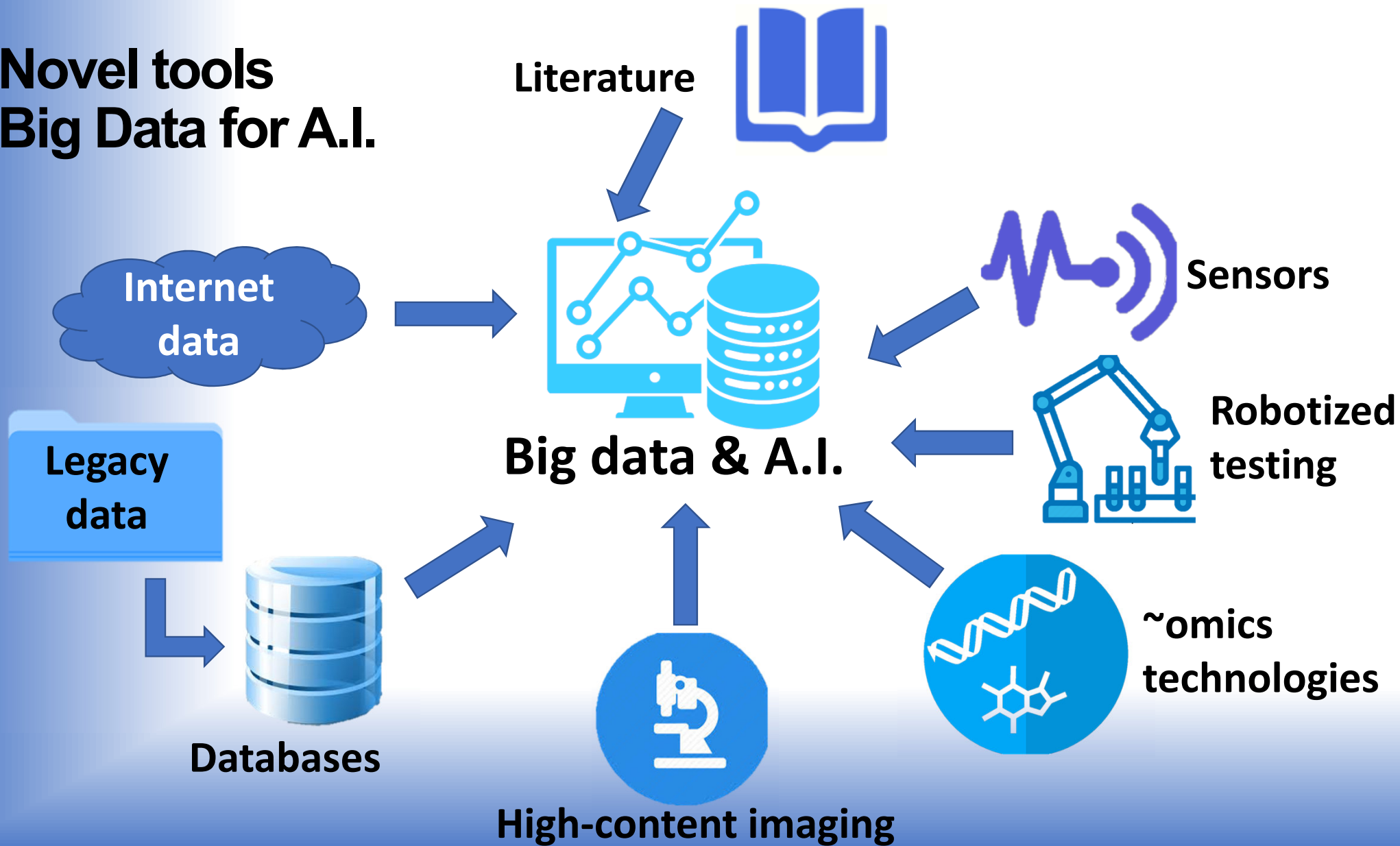
*(Source: Forbes)*



© Statista 2021

**84% of data in the world were created in the last 6 years**  
Volume of data/information created, captured, copied, and consumed worldwide from 2010 to 2025 *(in zettabytes)*

# Novel tools Big Data for A.I.





# Digital pathology

Many (pathologists) see toxicology as the pathology caused by chemicals

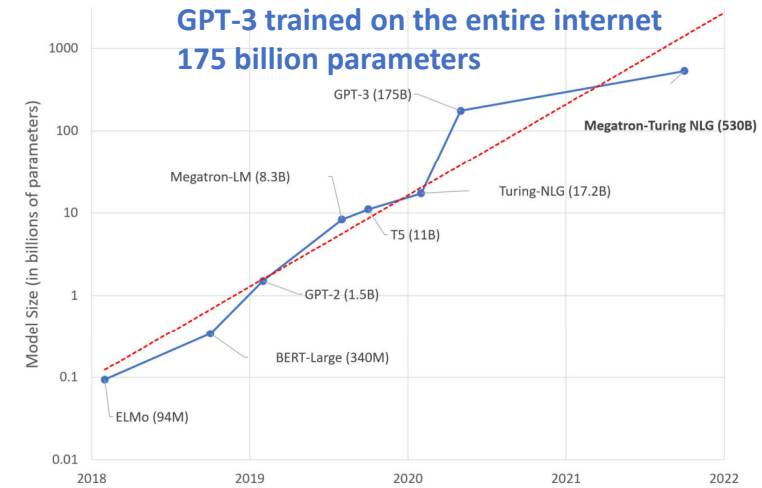
Cartoon of traditional vs. modern pathologist

Image analysis  
High-content imaging  
Cloud storage  
Standardized  
interpretation

Symbol NLP

Data extraction from literature, reports & databases

Picture reading robot



Picture robot with tools

## **AI works through**

- **Big data**
- **Deep learning gets much better with more data**
- **Handles data gaps and redundancies**
- **Network effects**
- **Transfer learning**
- **Data curation not important**
- **Humans in the loop (supervised and reinforcement learning)**
- **Increasingly explainable**

Symbol AI



Tom Luechtefeld

9 most common toxicity tests  
190,000 chemical's hazard  
cross-validation:  
87% correct

<https://sfmagazine.com/technotes/february-2019-wipo-u-s-and-china-lead-the-world-in-ai-innovation/>

ACCEPTED MANUSCRIPT

## Machine learning of toxicological big data enables read-across structure activity relationships (RASAR) outperforming animal test reproducibility



Thomas Luechtefeld, Dan Marsh, Craig Rowlands, Thomas Hartung ✉

*Toxicological Sciences*, kfy152, <https://doi.org/10.1093/toxsci/kfy152>

Published: 11 July 2018



TOXICOLOGY Science, 12 Feb 2016

### *A crystal ball for chemical safety*

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

nature  
Home | News & Comment | Research | Careers & Jobs | Current Issue | Archive | Audio & Video | For Advertisers

News & Comment | News | 2016 | February | Article

NATURE | NEWS

#### Legal tussle delays launch of huge toxicity database

Health risks of nearly 10,000 chemicals charted to help predict toxicity of untested substances.

Natalie Gillett

nature  
Home | News & Comment | Research | Careers & Jobs | Current Issue | Archive | Audio & Video | For Advertisers

News & Comment | News


NEWS | 11 JULY 2018

#### Software beats animal tests at predicting toxicity of chemicals

Machine learning on vast trove of safety data improves automated assessments.

Science Home News Journals Topics Careers Advertisement

Login | My account | Contact us



An estimated 3 million to 4 million rabbits, rats, and other animals are used annually around the world for chemical safety tests. CARMEL DORRIS/ISTOCK PHOTO.

### New digital chemical screening tool could help eliminate animal testing

By Vanessa Zaitzinger | Jul 11, 2018 | 11:00 AM

# Ongoing RASAR developments

**79% (n=131) and 80% (n=375) accuracy in predicting HUMAN skin sensitization (Golden et al., ALTEX, 2020)**

**38,250 predictions for 4,729 food-relevant substances  
83% accurate (n=139) (Fu et al., 2022)**

**Preliminary (Luechtefeld et al., in preparation):**

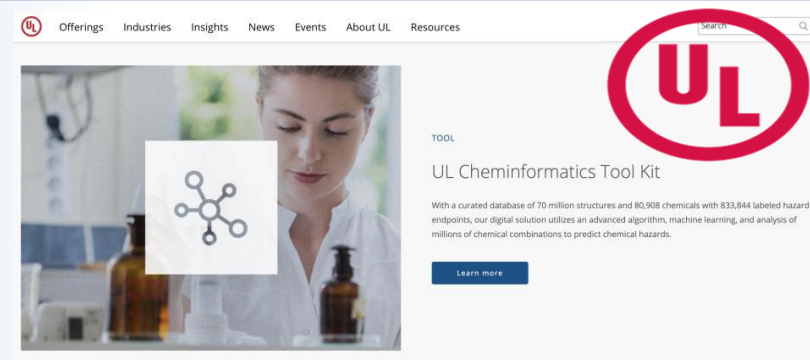
**Reproductive Tox 82% accurate (n=1152)**

**Carcinogenicity 75% accurate (n=950)**

**Androgen effect 98% accurate (n=8492)**

**Estrogen transactivation 80% accurate (n=1660)**

**EU ONTOX project (\$20 million, 2021-2026) to expand to liver, kidney and developing brain**



**Accepted for Australian Industrial  
Chemical Legislation 2020**

**Illustration limit is the sky**

<https://www.dreamstime.com/photos-images/sky-limit.html>



# Green toxicology

– the toxicology aspects of green chemistry



Alex Maertens

## Another use of alternatives methods

Green Chemistry Series

## Green Toxicology

Making Chemicals Benign by Design

Alexandra Maertens

TOXICOLOGICAL SCIENCES, 161(2), 2018, 285–289

Sci  
Years

doi: 10.1093/toxsci/kfx243

Advance Access Publication Date: December 18, 2017

Editorial

ly About and Avoid Toxic

ng\*,†,1

*Finding alternative*

*Chemicals*

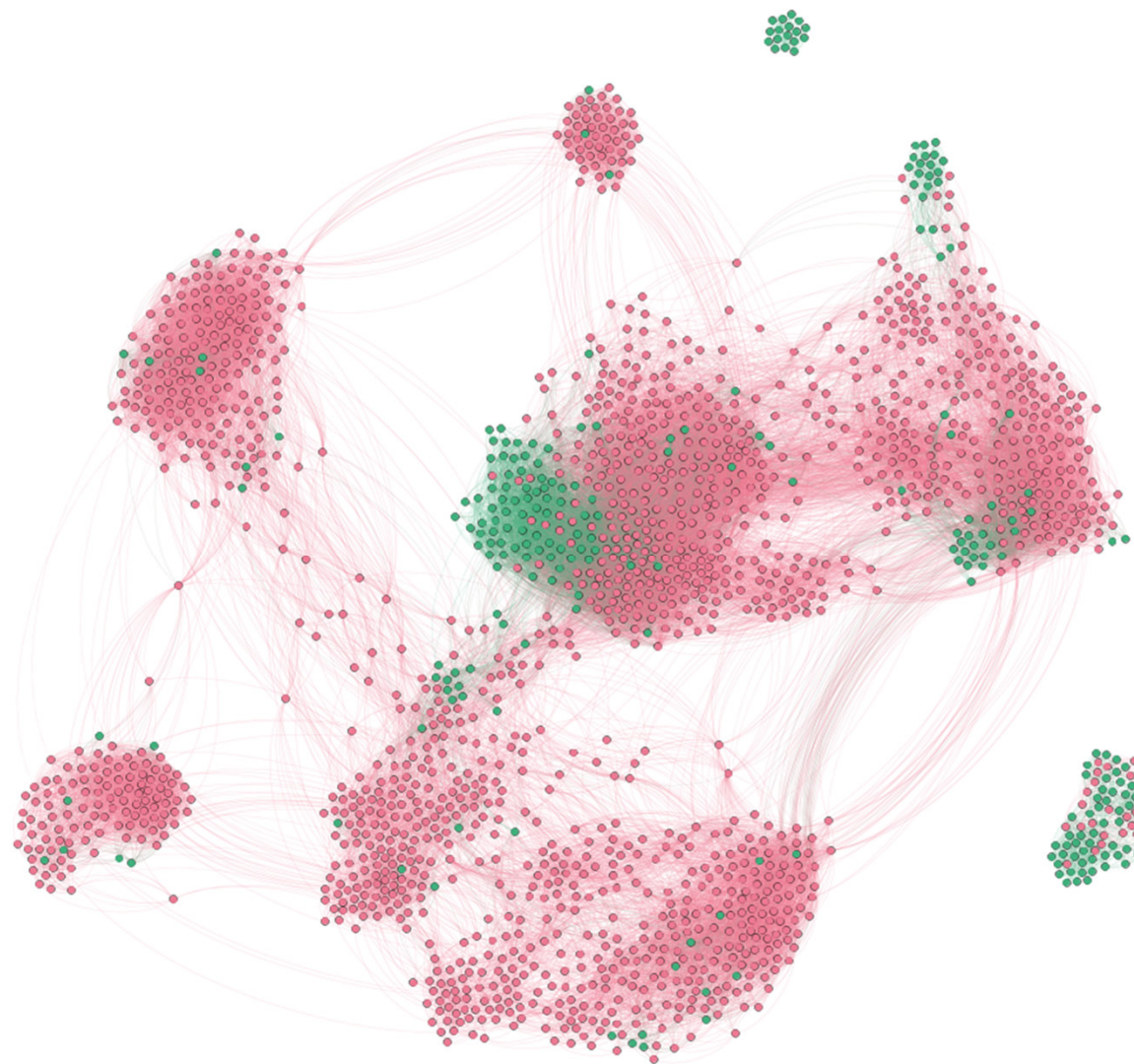
**Example**

**Dichloromethane**

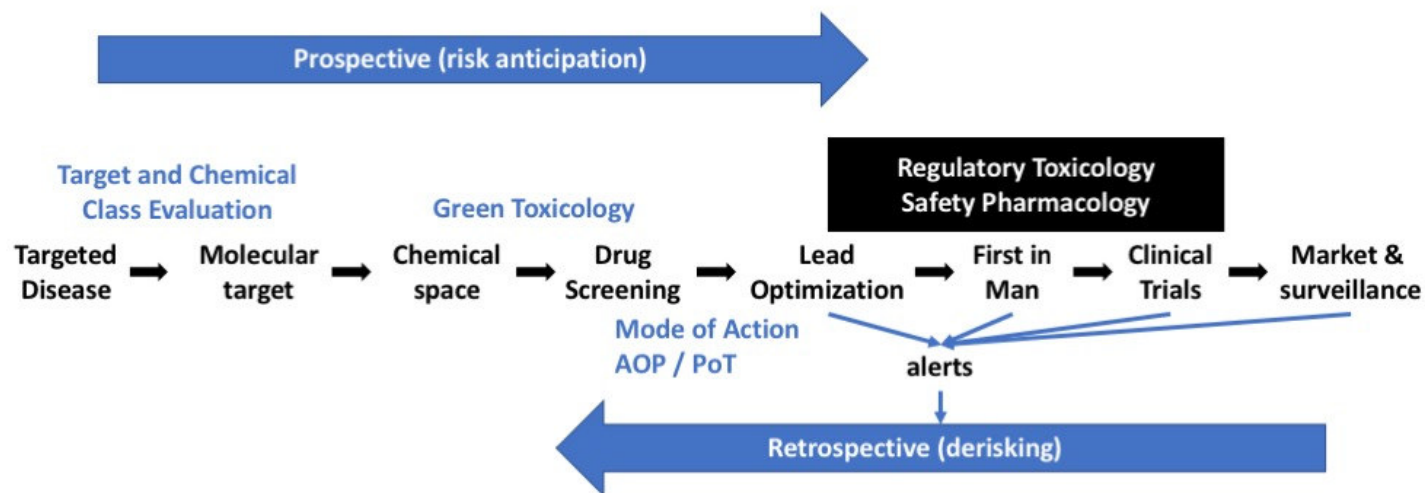
**1. Tox space**

**2. Chemical  
Similarity Space**

**3. Optimized  
Combination**



# Investigative (Drug) Toxicology



From destructive  
to constructive  
toxicology!

## t4 Workshop Report

### Optimizing Drug Discovery by Investigative Toxicology: Current and Future Trends

Mario Beilmann<sup>1,§</sup>, Harrie Boonen<sup>2,§</sup>, Andreas Czich<sup>3,§</sup>, Gordon Dear<sup>4,§</sup>, Philip Hewitt<sup>5,§</sup>, Tomas Mow<sup>6,§</sup>, Peter Newham<sup>7,§</sup>, Teija Oinonen<sup>8,§</sup>, Francois Pognan<sup>9,§</sup>, Adrian Roth<sup>10,§</sup>, Jean-Pierre Valentin<sup>12,§</sup>, Freddy Van Goethem<sup>13,§</sup>, Richard J. Weaver<sup>14,§</sup>, Barbara Birk<sup>15</sup>, Scott Boyer<sup>16</sup>, Francesca Caloni<sup>17</sup>, Alice E. Chen<sup>18</sup>, Raffaella Corvi<sup>19</sup>, Mark T. D. Cronin<sup>20</sup>, Mardas Daneshian<sup>21</sup>, Lorna C. Ewart<sup>7</sup>, Rex E. FitzGerald<sup>22</sup>, Geraldine A. Hamilton<sup>23</sup>, Thomas Hartung<sup>21,24</sup>, Joshua D. Kangas<sup>25</sup>, Nynke I. Kramer<sup>26</sup>, Marcel Leist<sup>21</sup>, Uwe Marx<sup>27</sup>, Sebastian Polak<sup>28,29</sup>, Costanza Rovida<sup>21</sup>, Emanuela Testai<sup>30</sup>, Bob van de Water<sup>31</sup>, Paul Vulto<sup>32</sup> and Thomas Steger-Hartmann<sup>11,§</sup>



ALTEX 2019, 36:289-313

# Human cell and tissue culture

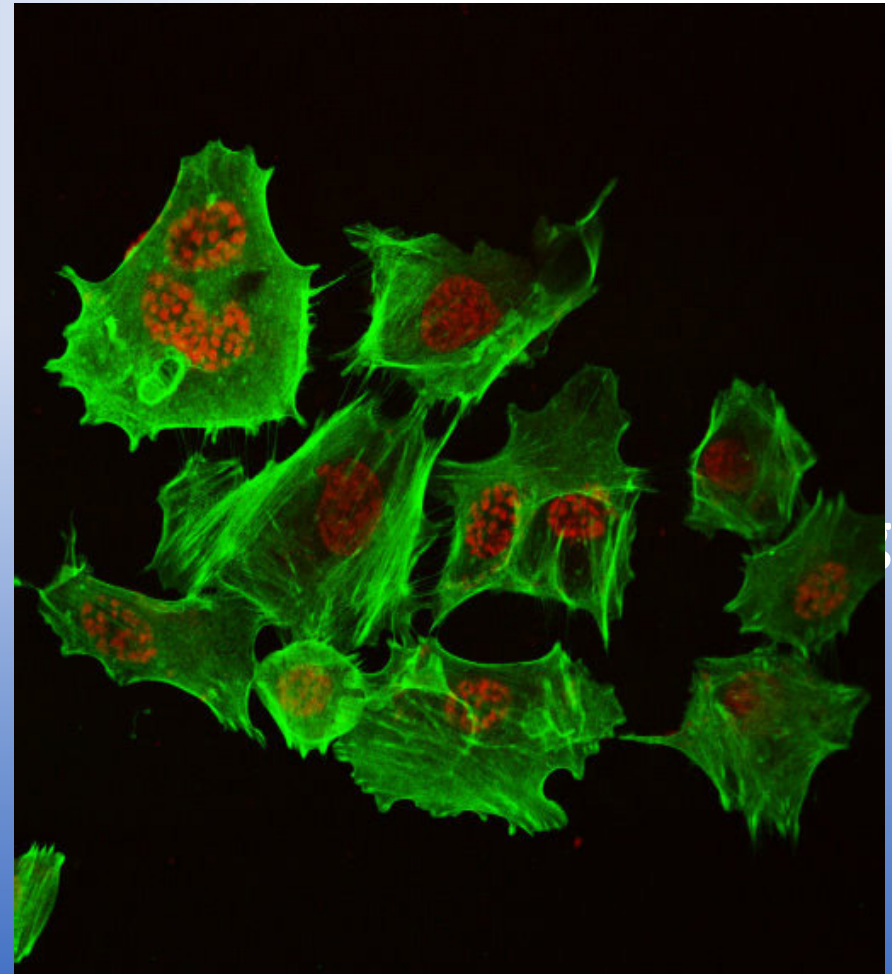
## Irreprodu-*cell*-bility

Primary cells of  
limited access,  
quality, and  
quantity

Symbol game changer

Tumor cell lines

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



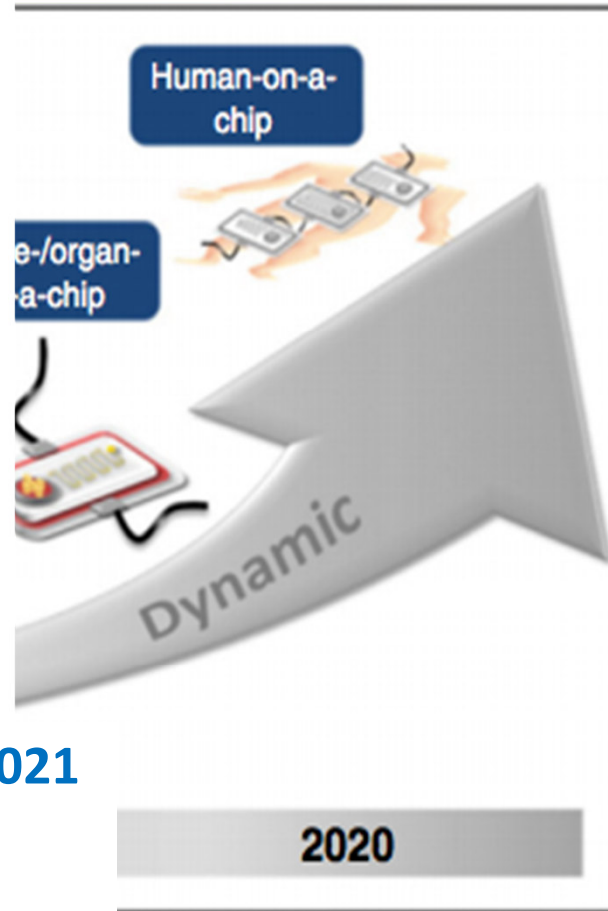
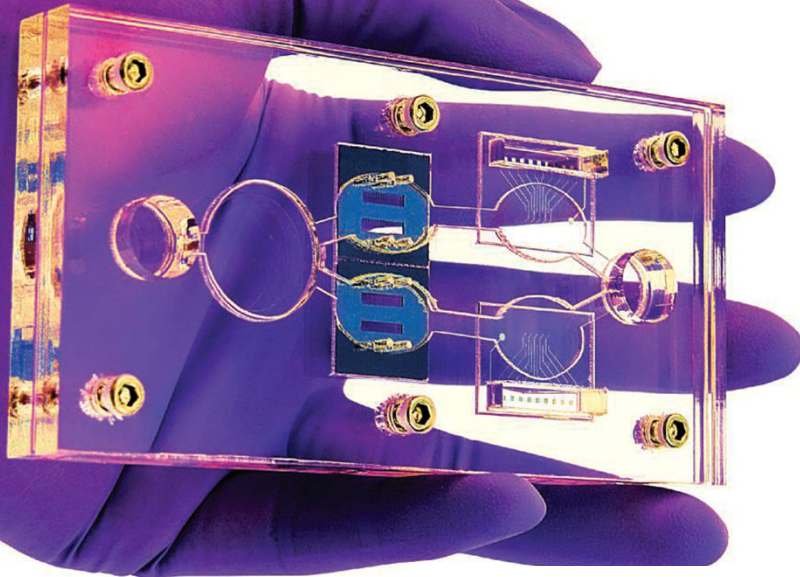
tical



# Evolution of Cell Culture - high-tech & business opportunity

## INSIGHTS

Microfluidic systems can connect multiple types of human tissues and mimic aspects of human physiology to improve evaluations of drug responses.



PERSPECTIVES

MEDICINE

## Human microphysiological systems for drug development

Organs-on-chips could be used to assess drug efficacy and support personalized medicine

Science 16 Sep 2021



Marx et al., Biology-inspired micro-physiological system approaches to solve the prediction dilemma of substance testing using animals. ALTEX 2016, 33:272-321.



Marx et al., Biology-inspired microphysiological systems to advance medicines for patient benefit and animal welfare. ALTEX 2020, 37:365-394 .



2020

Current Opinion in Biotechnology





**MPS WORLD SUMMIT**  
CONNECT, EXCHANGE, EDUCATE

**New Orleans 30 May-3 Jun '22**

**Hosts: Suzie Fitzpatrick, FDA**

**Thomas Hartung, Hopkins**

**Don Ingber, Harvard**



**<https://mpsworldsummit.com>**

**52 organizations**

**34 Scientific Advisory Board**

**665 Registered (215 Online, 65 FDA)**

**26 Countries**

**142 speakers, 189 posters**

**\$450k from NCATS**

**Forming the International MPS  
Society and Conference Series**

# 2<sup>nd</sup> MPS world Summit: June 26-30<sup>th</sup> 2023



Illustration quality > quantity

- Quality of cell model (GCCCP)
- Quality of reporting (GIVReSt)
- Quality of results (validation)

Illustration “finally”



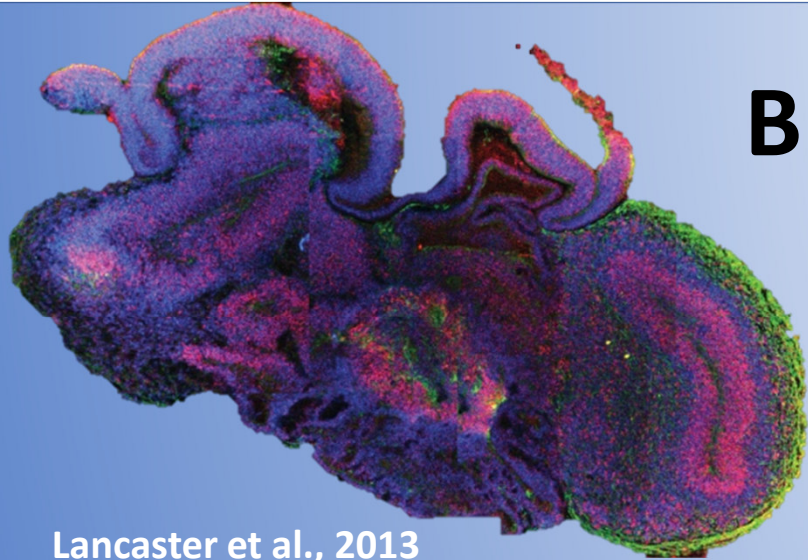
**Guidance Document on Good Cell and Tissue Culture  
Practice 2.0  
(GCCP 2.0)**

**ALTEX 2022, 39:30-70**

*David Pamies<sup>1</sup>, Marcel Leist<sup>2,3</sup>, Sandra Coecke<sup>4</sup>, Gerard Bowe<sup>4</sup>, Dave Allen<sup>5</sup>, Gerhard Gstraunthaler<sup>6</sup>, Anna Bal-Price<sup>4</sup>, Francesca Pistollato<sup>4</sup>, Rob deVries<sup>7,8</sup>, Helena T. Hogberg<sup>9</sup>, Thomas Hartung<sup>2,9</sup> and Glyn Stacey<sup>10,11,12</sup>*



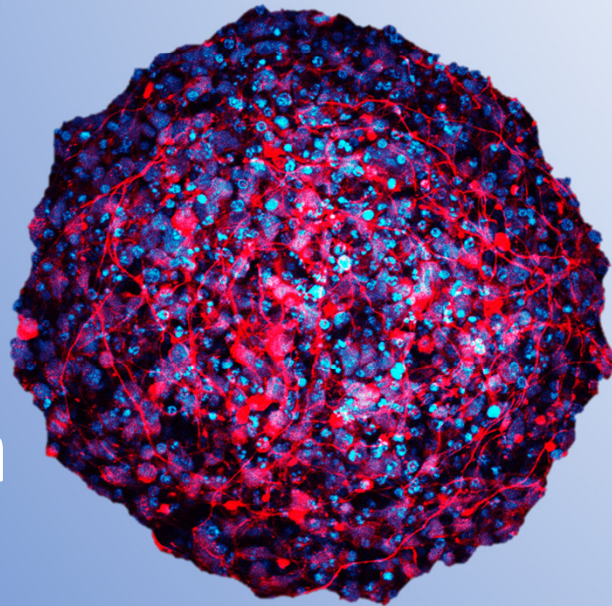
# Brain Organoids



Lancaster et al., 2013

Autism  
COVID-19  
Glioblastoma

....



2016

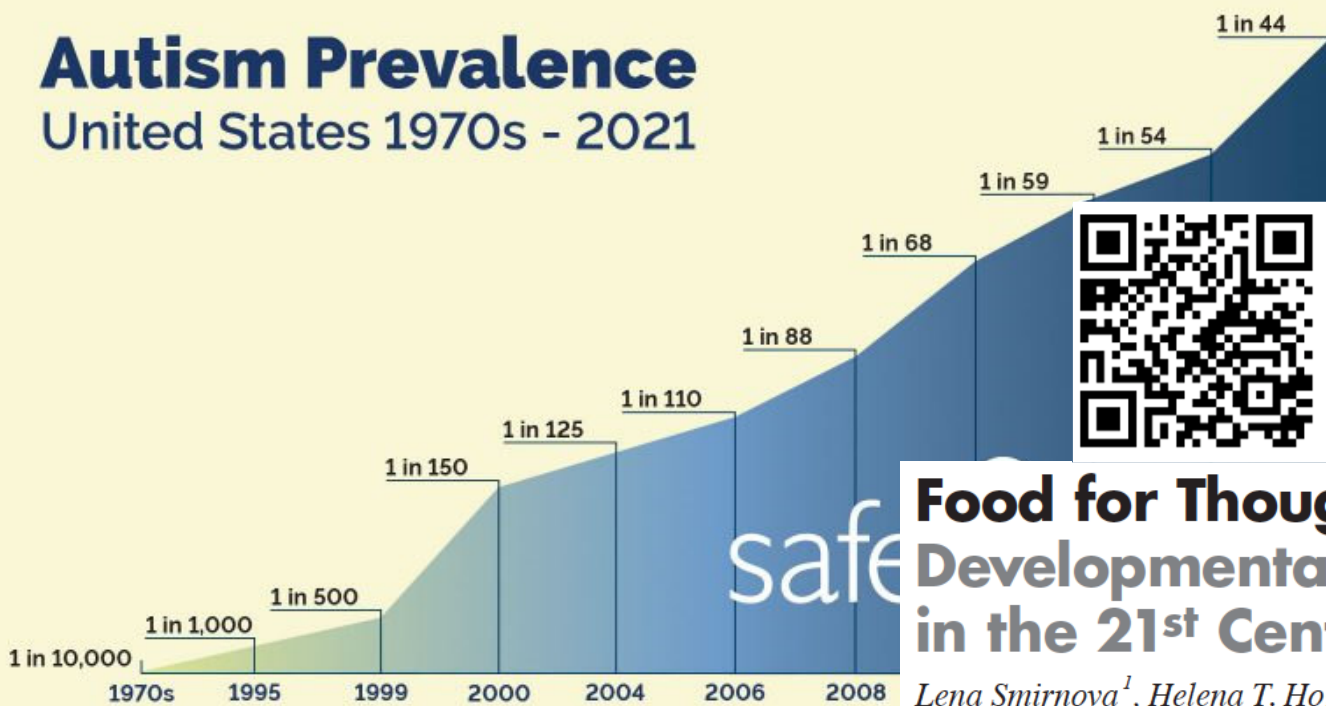






# IS DEVELOPMENTAL NEUROTOXICITY INVOLVED?

## Autism Prevalence United States 1970s - 2021



Embryo with chemical in brain

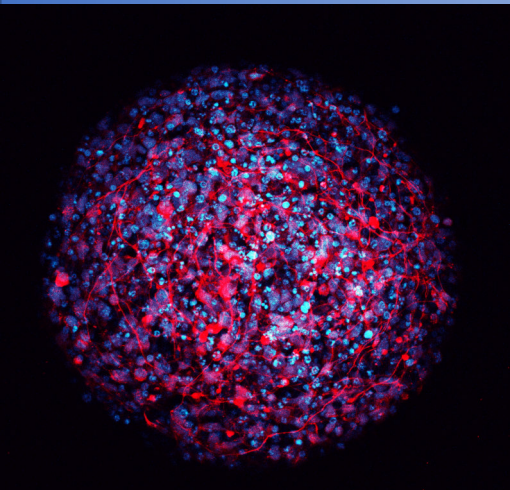


safe

### Food for Thought ...

### Developmental Neurotoxicity – Challenges in the 21<sup>st</sup> Century and *In Vitro* Opportunities

Lena Smirnova<sup>1</sup>, Helena T. Hogberg<sup>1</sup>, Marcel Leist<sup>2</sup>, and Thomas Hartung<sup>1,2</sup>



# DNT in human brain organoids

 frontiers **Front Cell Neurosci 2020**

## Antidepressant Paroxetine exerts developmental neurotoxicity in an iPSC-derived 3D human brain model

Xiali Zhong<sup>1,2</sup>, Georgina Harris<sup>1</sup>, Lena Smirnova<sup>1</sup>, Valentin Zufferey<sup>3</sup>, Rita Sa<sup>4</sup>, Fabiele Baldino Russo<sup>5</sup>, Patricia C. Baleeiro Beltrao Braga<sup>5</sup>, Megan Chesnut<sup>1</sup>, Marie-Gabrielle Zurich<sup>3</sup>, Helena Hogberg<sup>1</sup>, Thomas Hartung<sup>6,7</sup>, David Pamies<sup>3,1\*</sup>



**Tox Appl Pharmacol 2018**



Toxicology and Applied Pharmacology

journal homepage: [www.elsevier.com/locate/taap](http://www.elsevier.com/locate/taap)

## Rotenone exerts developmental neurotoxicity in a human brain spheroid model

David Pamies<sup>a</sup>, Katharina Block<sup>a</sup>, Pierre Lau<sup>b</sup>, Laura Gribaldo<sup>b</sup>, Carlos A. Pardo<sup>c</sup>, Paula Barreras<sup>c</sup>, Lena Smirnova<sup>a</sup>, Daphne Wiersma<sup>a</sup>, Liang Zhao<sup>a,d</sup>, Georgina Harris<sup>a</sup>, Thomas Hartung<sup>a,e</sup>, Helena T. Hogberg<sup>a,\*</sup>

Archives of Toxicology  
<https://doi.org/10.1007/s00204-020-02903-2>

**Arch Toxicol 2021**

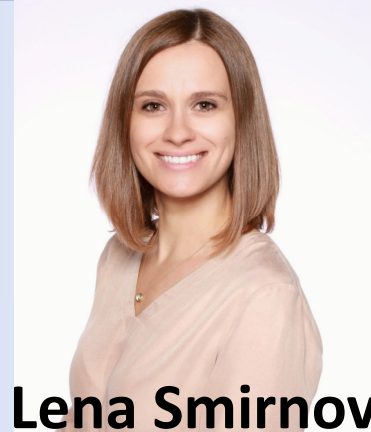


**ORGAN TOXICITY AND MECHANISMS**

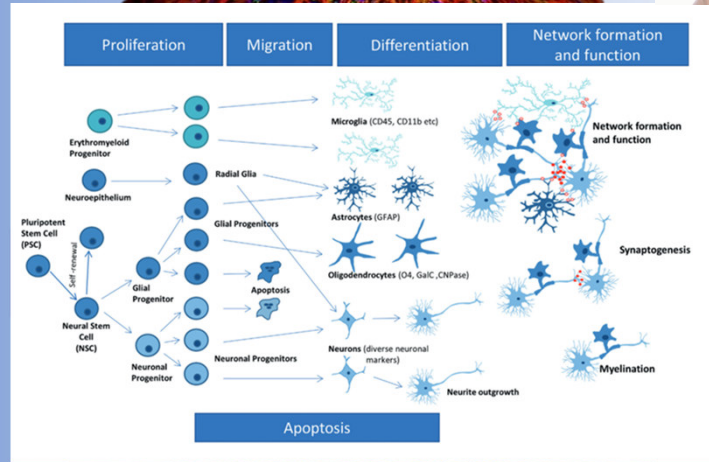
## Organophosphorus flame retardants are developmental neurotoxicants in a rat primary brainsphere in vitro model



# 6-in-1 BrainSphere assay to test Neurodevelopment



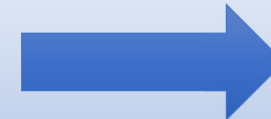
Lena Smirnova



Bal-Price et al., 2018a

- Neuronal differentiation
- Myelination
- Neurite outgrowth
- Synaptogenesis
- Glia migration & Gliosis
- Neural network (E-phys)

CRISPR/CAS9



Reporter/  
Fusion  
proteins



Mini- Brainbow

Neurons

Astrocytes

Oligodendrocytes

Synapses

3D electrophys



High content imaging  
Toxicant/drug screening

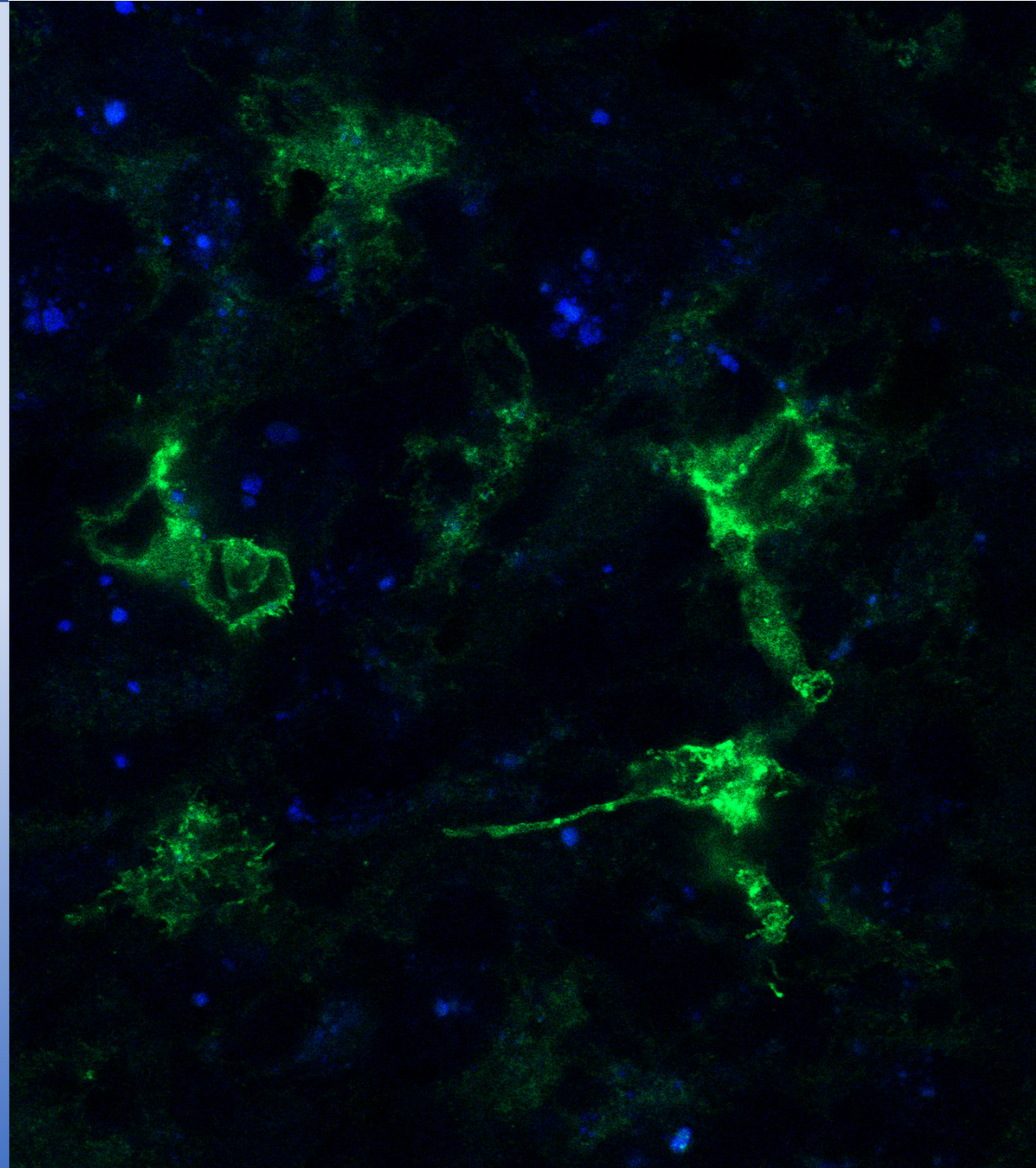




# CRISPR/Cas9 Knock-Ins

Oligodendrocytes (PLP-GFP)

Synapsis (Synaptophysin-BFP)

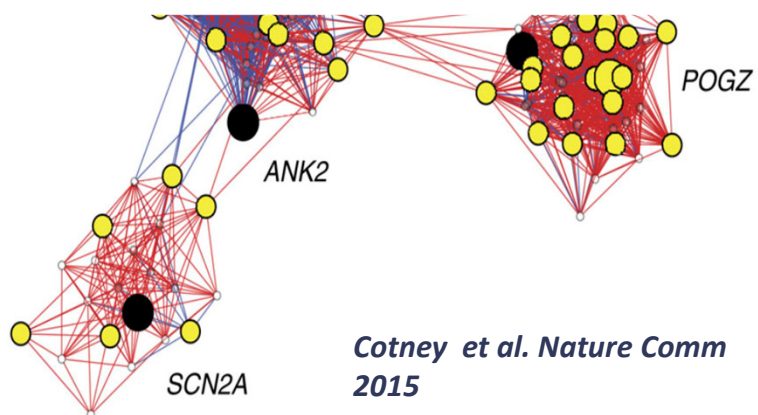


# Bloomberg School Researchers Awarded \$11.7 Million Five-Year NIH Grant to Build and Lead Autism Center of Excellence Network

Published **September 08, 2022**

DISABILITY

Network will aggregate global research projects studying gene-environment interaction to understand autism's causes and to improve quality of life among autistic individuals



**Lena Smirnova**

Functional and Molecular  
signatures





# BRAIN ORGANOIDS

STANDARDIZED  
HUMAN 3D  
DEVELOPMENT  
FROM IPSC



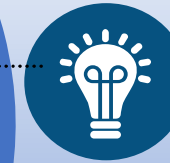
GLIA CELLS,  
MYELINATION  
+ ADDED MICRO-  
GLIA



INFECTION  
CANCER GRAFTS  
TOXICITY



GENETIC  
BACKGROUNDS,  
+ RISK GENES,  
REPAIR



+ REPORTER GENES  
GENE X

ENVIRONMENT  
MIXTURES  
FUNCTIONAL



ASSAYS:

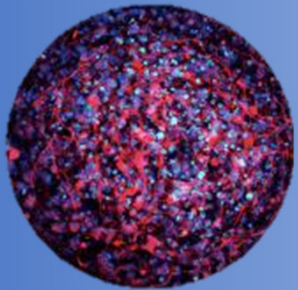
NEURITES,  
SYNAPSES, 3D  
ORGANOID



LEG  
INTELLIGENCE  
(O.I.)

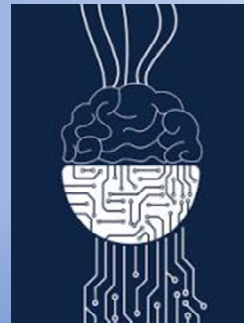
# Organoid Intelligence (O.I.)

Discovery Grant



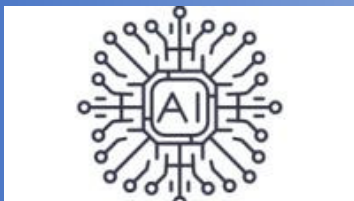
Brain Organoid

O.I.



Input

Output



A.I.



Human brain organoid caged in shell electrodes

Workshop 2022  
Baltimore Declaration Toward OI

**What is emerging that can help us?**

**Exposure science** (high throughput and untargeted exposomics, remote sensing, citizen science ...)

**Technologies** (~omics, high-throughput, MPS, A.I.)

**Evidence Integration** (Evidence-based Tox, IATA, Green Tox Investigative Tox, Mechanistic Validation, Probabilistic Risk Assessment, Systems Toxicology, virtual experiments...)

# The challenge

Illustration data integration

[https://www.loopclosed.com.au/program\\_services/data\\_integration\\_and\\_analysis.html](https://www.loopclosed.com.au/program_services/data_integration_and_analysis.html)

## Similar for

- Systematic reviews
- Risk assessments
- Integrated Testing Strategies

Illustration putting puzzle together

<http://phd.dia.uniroma3.it/multi-source-data-integration-with-humans-in-the-loop/>

# *In vivitrosi*

Replacement of animal testing by integrated approaches to testing and assessment (IATA): a call for in vivitrosi

Francesca Caloni<sup>1</sup>  · Isabella De Angelis<sup>2</sup> · Thomas Hartung<sup>3,4</sup>

Arch Toxicol 2022



**Aka Integrated  
Testing Strategies,  
IATA, Defined  
Approaches...**

**1 + 1 > 2**



# EVIDENCE INTEGRATION: Evidence-based Toxicology, systematic reviews, risk assessment...

## 2006 Article

Human & Experimental Toxicology (2006) 25: 497–513  
www.sagepublications.com

### Toward an evidence-based toxicology

S Hoffmann\* and T Hartung

European Commission, JRC – Joint Research Centre, Institute for Health & Consumer Protection, ECVAM – European Centre for the Validation of Alternative Methods, 21020 Ispra (VA), Italy



## 2007 Conference

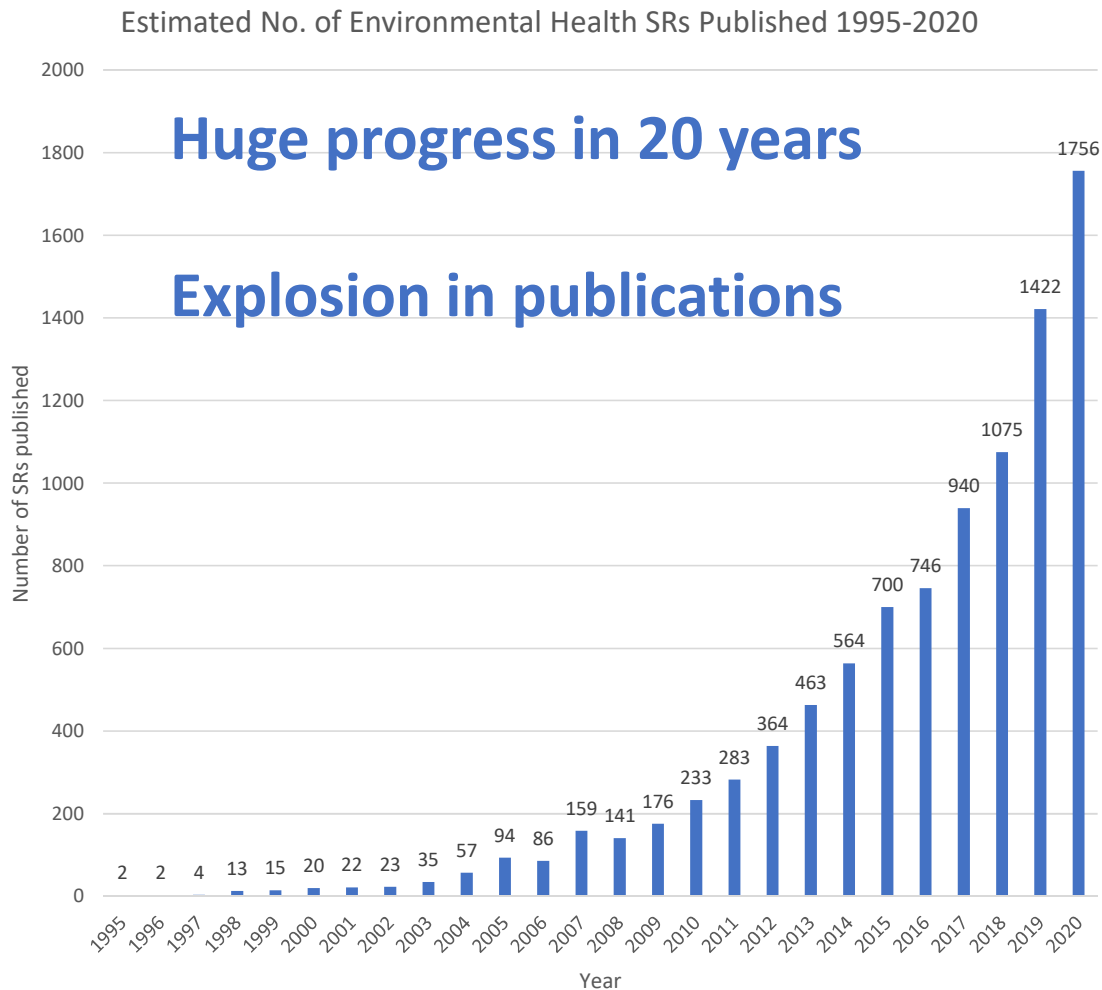


## 2009 Chair Hopkins

2011 Organization  
[www.ebtox.org](http://www.ebtox.org)

**ebtcc**  
Evidence-based Toxicology Collaboration

# The explosion of systematic reviews



**ebtc**  
Evidence-based Toxicology Collaboration



**Katya Tsaion**

- ~1000 people engaged with EBTC in 12 years
- Stakeholders at every level, everywhere
- EBT journal from 2023
- EBT association forming

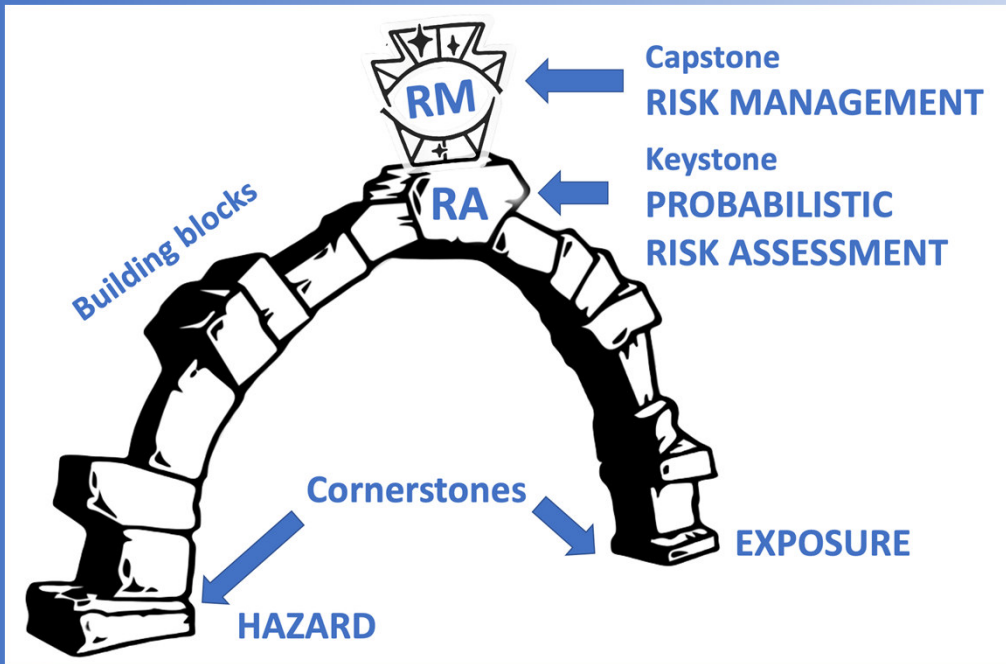
ALTEX 2022

"Probability is the very guide of life."  
Cicero (106 – 43 B.C.)

Food for Thought ...

# Probabilistic Risk Assessment – the Keystone for the Future of Toxicology

Alexandra Maertens<sup>1</sup>, Emily Golden<sup>1</sup>, Thomas H. Luechtefeld<sup>1,2</sup>, Sebastian Hoffmann<sup>1,3</sup>,  
Katya Tsaoun<sup>1</sup> and Thomas Hartung<sup>1,4</sup>



Workshop 4-6 July 2022  
Ranco, Italy

Chemicals  
Mixtures /products  
Metabolites

X

Humans  
Animals  
Ecosystems

X

Uses

X

Industries  
= legislations

X

Countries  
Regions

=

Tons of test needs  
Tons of discrepancies  
= Mission Impossible

Mixture  
Tox

One  
Health  
Cumulative  
Exposure  
OSOA

Harmonization

Exposomics, EI  
Exposure science, HT exposure



Prob Exposure



ProbRA



Prob Hazard

Evidence  
generation

Evidence  
integration

Legacy  
data

MPS,  
AI, HTS

Evid-based Tox  
Test strategies, AI

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

John Maynard Keynes

(1883 - 1946)

Picture breaking out of flat world

Slides available:

