

21st century alternative methods for 21st century safety sciences

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



**A CENTURY OF SAVING LIVES
MILLIONS AT A TIME**

**JOHNS HOPKINS
BLOOMBERG SCHOOL
OF PUBLIC HEALTH**



BraCVAM

**Centro Brasileiro
para Validação de Métodos
Alternativos**

*Brazilian Center for Validation
of Alternative Methods*



1993 Baltimore, ... 2002 New Orleans, ...
2011 Montreal, ... 2017 Seattle, ...



**CANADIAN
CENTRE FOR
ALTERNATIVES TO
ANIMAL
METHODS**

**CANADIAN
CENTRE FOR THE
VALIDATION OF
ALTERNATIVE
METHODS**

PAN-AMERICAN
Conference for Alternative Methods

2016 Baltimore
2018 Rio de Janeiro
2020 Canada



1981



Stakeholder Platform



2010



Transatlantic Hub



2012



CAAT EU Policy Program



Our team



- Big Data and Read-across
- Green Toxicology
- Evidence-based Toxicology
- Collaboration
- Good Cell Culture Practice
- Human-on-a-Chip - Microphysiological Systems
- Human Toxome
- Refinement
- Information and Communications
- Education
- Grants

Thanking our sponsors (industry, philanthropy, agencies)

Current



...and individuals



Recent



Conflict of Interest Statement



**Founder
(organoids)**

ORGANOME

AstraZeneca  **Consultant**



**Consultant
Computational
Toxicology**



3/2018

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ISSN 1868-596X
273-432 (2018)

ALTEX

ALTERNATIVES TO ANIMAL EXPERIMENTATION

Food for thought ...
Lucy Meigs, Lena Smirnova,
Costanza Rovida et al.
**Animal testing and
its alternatives –
the most important omics
is economics**

t⁴ Workshop Report
Anna Bal-Price,
Helena T. Hogberg,
Kevin M. Crofton et al.
**Recommendation on
test readiness criteria
for new approach
methods in toxicology:
Exemplified
for developmental
neurotoxicity**

t⁴ Workshop Report
David Pamies, Anna Bal-Price,
Christophe Chesné et al.
**Advanced Good Cell
Culture Practice for human
primary, stem cell-derived
and organoid models as
well as microphysiological
systems**



Research Article
Barbara Birk, Alexander Stähle,
Mathias Meier et al.
**Investigation of ruminant
xenobiotic metabolism
in a modified rumen
simulation system (RUSITEC)**

Research Article
Freia E. Schmid,
Florian Groeber-Becker,
Stefanie Schwab et al.
**A standardized method
based on pigmented
epidermal models
evaluates sensitivity
against UV-irradiation**

Research Article
Andrey Paloznikov,
Irina Gazaryan,
Maxim Shkurnikov et al.
**In vitro and in silico liver
models: Current trends,
challenges and opportunities**

BenchMarks
Marcel Leist and Jan G. Hengstler
**Essential components
of methods papers**

Meeting reports
Covers



transatlantic think tank for toxicology

49 FFT articles

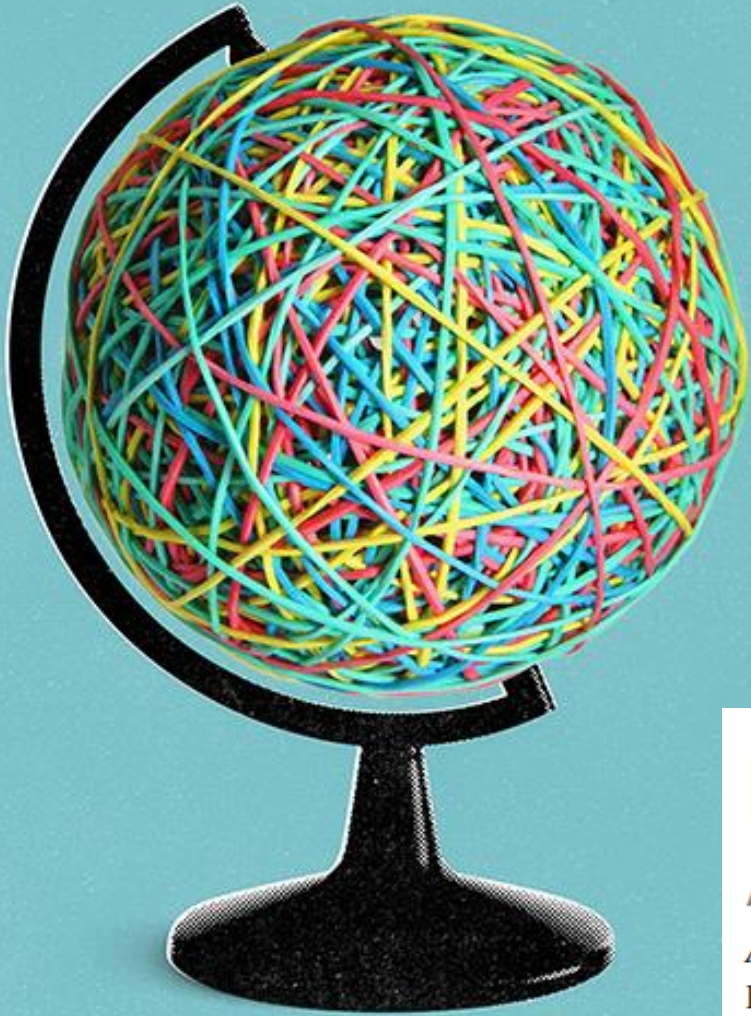
24 t⁴ workshop reports

11 t⁴ reports

Impact Factor 2016: 5.232

5-Year Impact Factor: 5.402

No alternative method will be used in a global industry until the last important market accepts it!



Food for Thought ... on Globalisation of Alternative Methods

Annamaria A. Bottini¹, Patric Amcoff² and Thomas Hartung³

EC Joint Research Centre, ¹ISD and ³IHCP/ECVAM, Italy and ²National Board of Agriculture, Sweden

Example

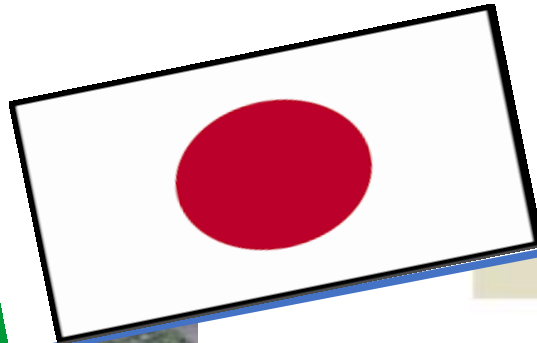
Two workshops 2011

Baltimore & ...

One key recommendation:

Broker International meeting of regulators to
abandon 1year dog study as done by EU and US

No new approach until the last important market
agrees.



on dogs

IN FOCUS NEWS

ology.

because facilities and test protocols
can be oversensitive to some com-
such as hormones, and their gastro-
system behaves differently to that
says Schmitt. He says that studies
dogs have proved to be poor models
published.

effort takes inspiration from an
opinioned more than a decade ago
ing expert David Smith, then
the London-based pharma-
of AstraZeneca and now at the
Animal Science Association,
UK. He brought together
etical companies and welfare
et discussions about dog test-
assessed the testing protocols
100 compounds and devel-
oped guidelines for dosage
(Regul. Toxicol. Pharmacol.
7). Smith says that this has
120 fewer dogs being used
year.

was no formal mechanism
ive efforts. CAAT is now
al framework. Before the
formed an international
aceutical companies to
for dog care and experi-

will be to do the same
ortunities, says Hartung.
panies will continue to
major region requires
through the European
reement for 12-month
dogs in 2006, the FDA

a new focus on dogs
change in animal
using fewer dogs.
dog-testing stand-
for over 60 years,"
improvements will
health as well."

ON THE BLOG



Genome could
help to fight
Tasmanian-
devil facial
tumours
guariter.com/111111

Food for Thought ... on the Economics of Animal Testing

Annamaria A. Bottini^{1,3} and Thomas Hartung^{2,4}

EU Joint Research Centre, Ispra, Italy, ¹ISD and ²IPSC / TRiVA, ³European School of Economics, Milan, Italy, ⁴Center for Alternatives to Animal Testing, Johns Hopkins University, Baltimore, USA



The Economics of Animal Testing

Annamaria A. Bottini¹ and Thomas Hartung²

¹Johns Hopkins University Medicine International, Baltimore, USA, and European School of Economics, Milan, Italy;

²Johns Hopkins University, CAAT, and CAAT-EU, University of Konstanz, Germany

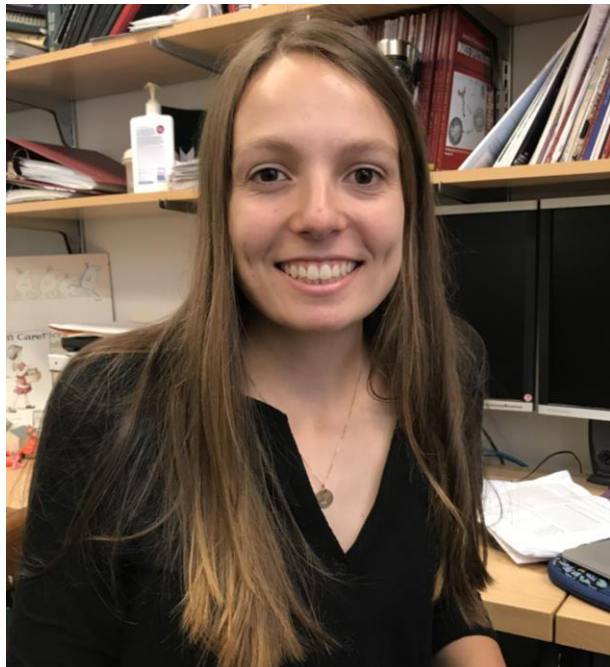
- **Economical reasons behind where we are, obstacles and opportunities**
- **Many methods are not worth their costs**
- **Transatlantic divide: animal welfare vs. new technologies**



Food for Thought ...

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

Lucy Meigs^{1,2}, Lena Smirnova², Costanza Rovida³, Marcel Leist³ and Thomas Hartung^{2,3}



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



**Traditional
Toxicology**

**Tox uses only 10%
of all animals,
but here 90% of
work on alternatives**



Toxicology

**\$3 billion per
year**

**\$20 million
per pesticide**



**\$1 million for
a cancer study**

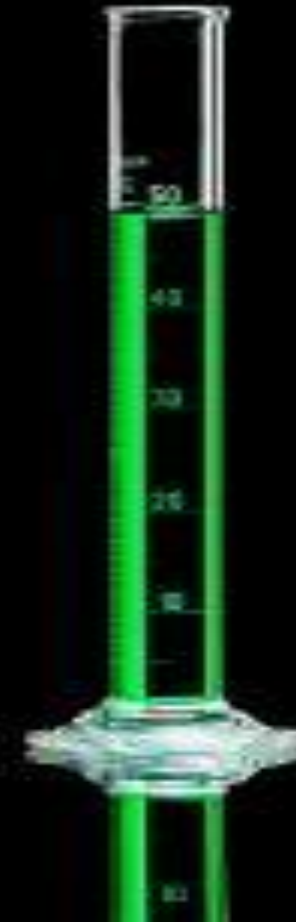
**About
5 years**

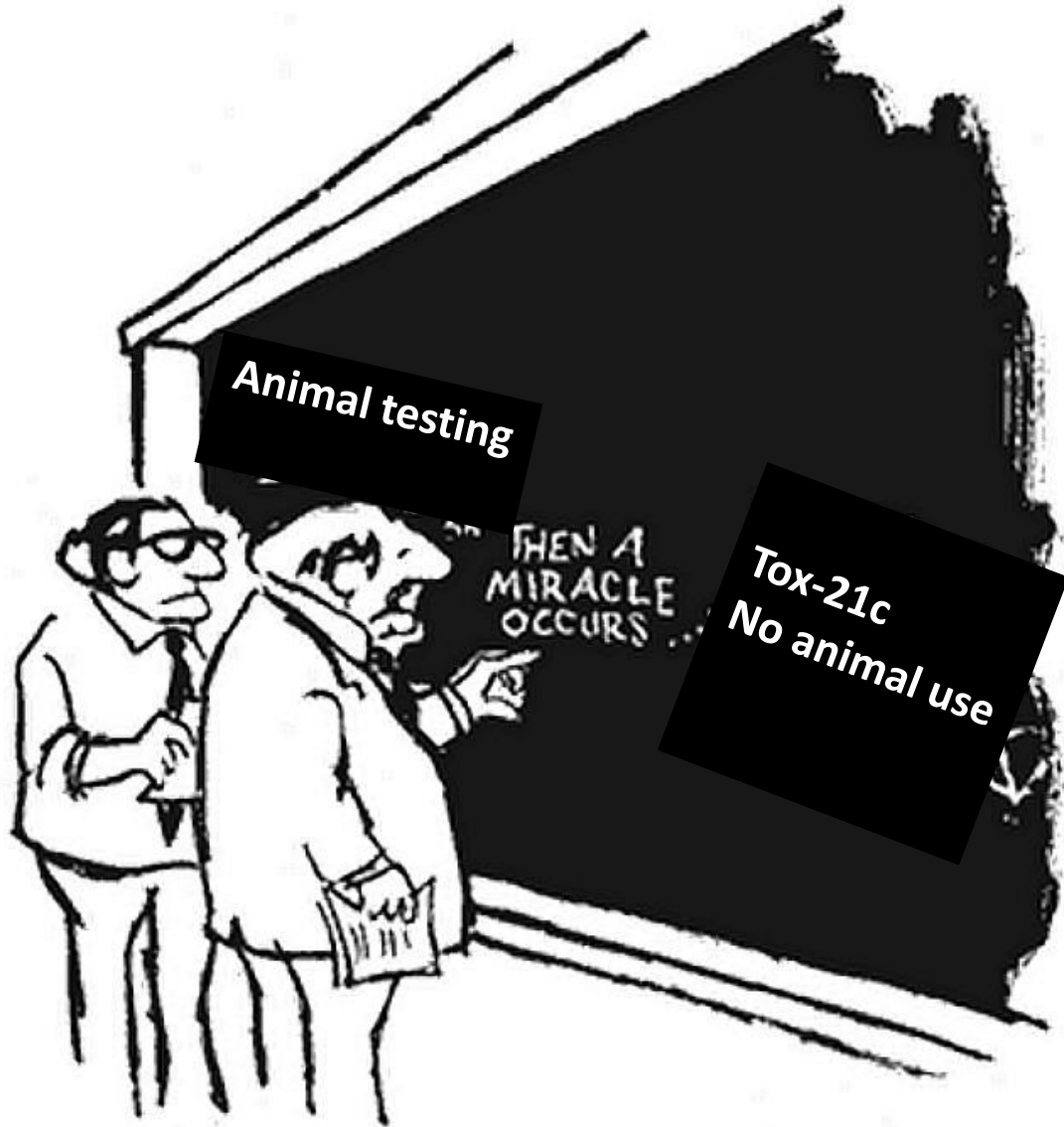
20kg needed

3% extensively tested
10% tested

After REACH (May 2018):
8% extensively tested
16% tested

140 million chemicals synthesized
140,000 in consumer products
1,000 new ones per year
(hardly tested)
40% market China





"I THINK YOU SHOULD BE MORE EXPLICIT HERE IN STEP TWO."



Clear strategies
and actual
implementation

**Animal tests in toxicology
should be better than
other areas:**

Standardized tests (OECD TG)

**Good Laboratory Practice
Skilled performers**

Maximum tolerated doses

**No disease models on top of
substance effects**



Six most frequent tox tests

Consuming 57% of animals in tox

350-750 chemicals with repeat tests

81% reproducible

69% reproducible for toxic chemicals

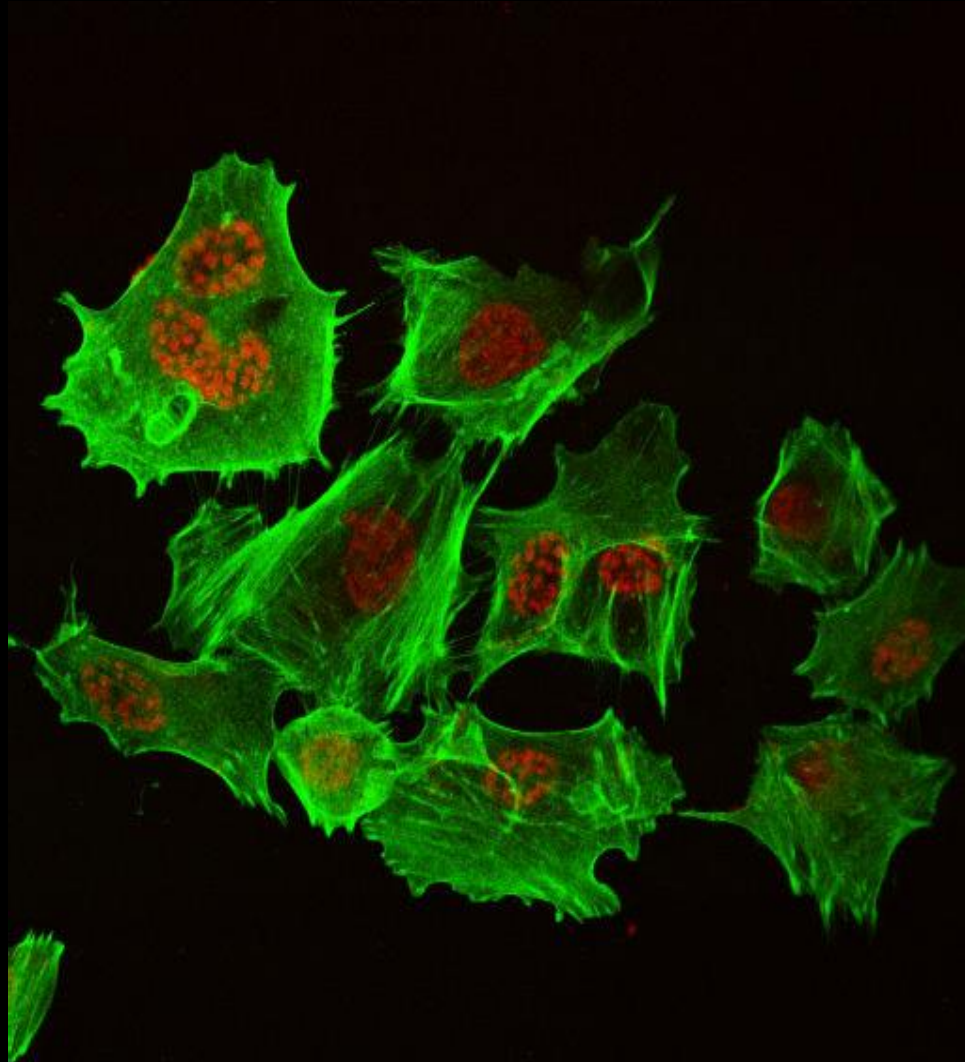




Not human-relevant
Will not change with
alternatives calibrated against
animals

Irreprodu-*cell*-bility

Cell tests have not less problems!



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

WORDS IN CLOUD: BILLION, PETABYTES, SYSTEM, SOFTWARE, LOGS, SEARCH, SCIENCE, EXAMPLES, SERVER, TECHNOLOGIES, MANAGEMENT, OPTIMIZE, STORAGE, RESEARCH, MASSIVELY, GOVERNANCE, ANALYSIS, SUPPORT, CAPTURE, MPP, INTERNET, COLLECTION, SETS, DATABASE, LARGE, EX, SERIES, DISK, BUSINESS, NETWORK, HUNDREDS, EXABYTE, USING TIME, TARGET, TYPES, BIG DATA CYCLE, PROCESS, VOLUME, ORGANIZE, DATABASE, LARGE, EX, SERIES, OPTIMIZE SUPPORT

**HOW TO ASSESS 140,000
CHEMICALS IN CONSUMER PRODUCTS?**

Data gap filling from similar chemicals



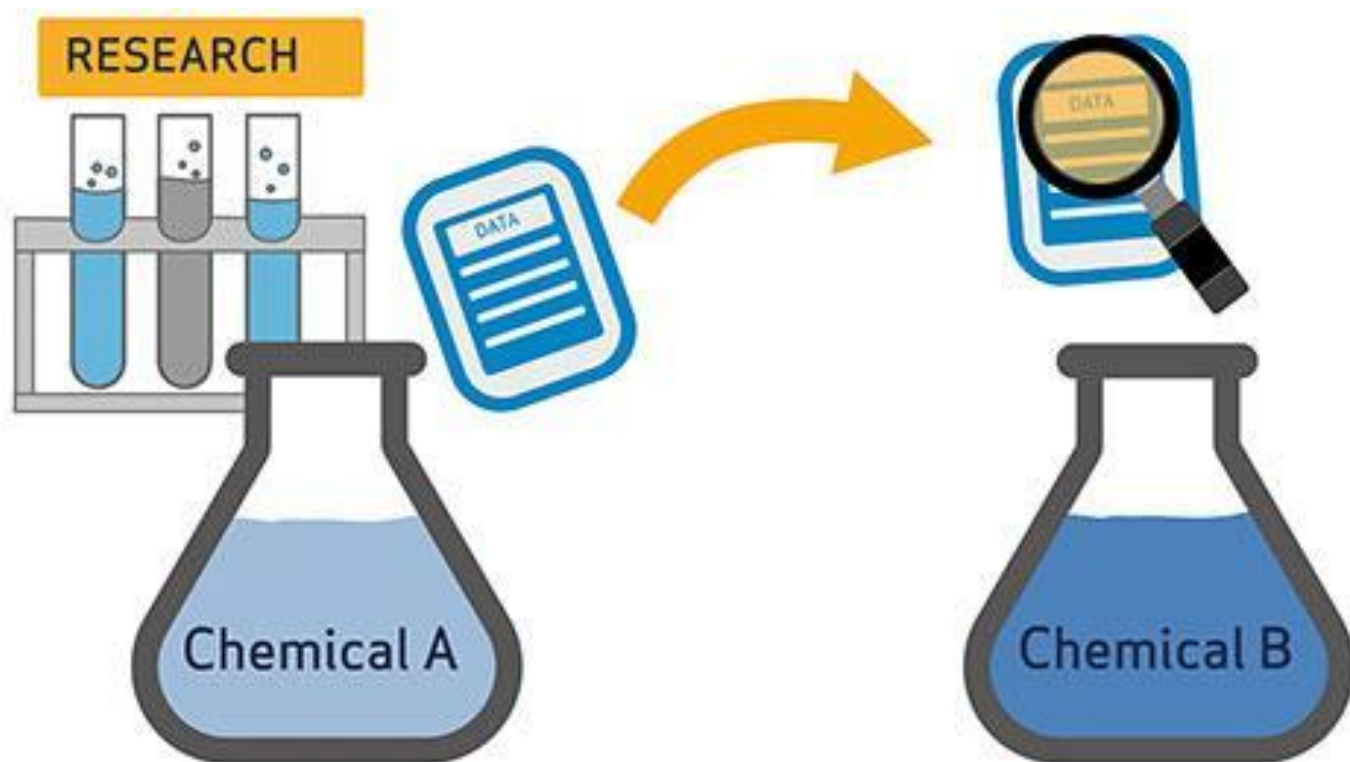
**Good Old Boys
Sat Around a Table**

*Traditional Read-Across has a
smell of GOBSAT*

- Simplistic identification of similar chemicals driven by data availability
- Good Read-Across Practice only emerging
- One-to-one or one-to-few read-across
- Cannot be validated

But it works and is broadly used in REACH!

CAAT Read-Across Program



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



t4 report*

Toward Good Read-Across Practice (GRAP) Guidance

Nicholas Ball^{1,8*}, Mark T. D. Cronin^{2*}, Jie Shen^{3*}, Karen Blackburn⁴, Ewan D. Booth⁵,
Mounir Bouhifd⁶, Elizabeth Donley⁷, Laura Egnash⁷, Charles Hastings⁸, Daland R. Juberg¹,
Andre Kleensang⁶, Nicole Kleinstreuer⁹, E. Dinant Kroese¹⁰, Adam C. Lee¹¹, Thomas Luechtefeld⁶,
Alexandra Maertens⁶, Sue Marty¹, Jorge M. Naciff⁴, Jessica Palmer⁷, David Pamies⁶, Mike
Penman¹², Andrea-Nicole Richarz², Daniel P. Russo¹³, Sharon B. Stuard⁴, Grace Patlewicz¹⁴,
Bernard van Ravenzwaay¹⁰, Shengde Wu⁴, Hao Zhu¹⁵ and Thomas Hartung^{6,15}



t4 report*

Supporting Read-Across Using Biological Data

Hao Zhu¹, Mounir Bouhifd², Elizabeth Donley³, Laura Egnash³, Nicole Kleinstreuer⁴,
E. Dinant Kroese⁵, Zhichao Liu⁶, Thomas Luechtefeld², Jessica Palmer³, David Pamies²,
Jie Shen⁷, Volker Strauss⁸, Shengde Wu⁹ and Thomas Hartung^{2,10}

Regulatory Acceptance of Read-Across: Report from an International Satellite Meeting at the 56th Annual Meeting of the Society of Toxicology

Megan Chesnut,¹ Takashi Yamada,² Timothy Adams,³ Derek Knight,⁴ Nicole Kleinstreuer,⁵ George Kass,⁶ Thomas Luechtefeld,¹ Thomas Hartung,^{1,7} and Alexandra Maertens¹

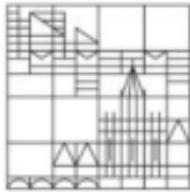
Megan Chesnut

Master of Health Sciences, May 2018





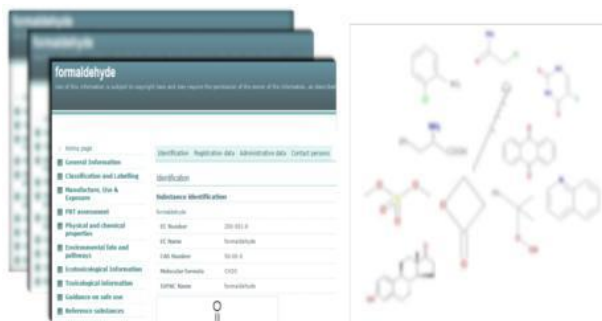
Universität
Konstanz



Think tank on “Read across as validated *in vitro* tool for regulatory toxicology“

Hotel Belvedere Ranco (Lago Maggiore), Italy (<https://bit.ly/2KvYOA0>)

16th to 18th July 2018



10,000 chemicals
800,000 tox
studies
(Dec 2014)



Natural language
processing
(Feb 2016)
&
Web app

TOXTRACK



Tom Luechtefeld



Nature online and
Scientific American

Initial irritation by EChA
Resolved in mtg. 4'2016
Led to data release 3'2017

Watch
CW+ **ChemicalRiskManager**
The hub for product safety resources

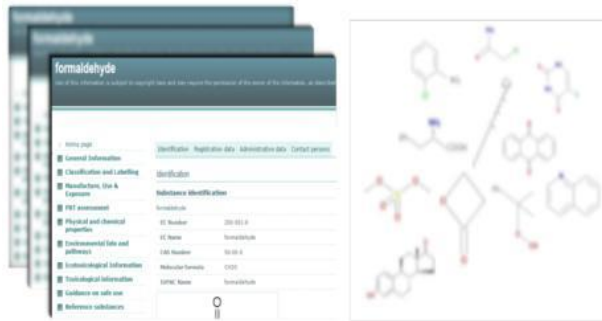
Chemical Watch
5 July 2017

News & features

Echa gives clarity on IP issues for Qsar predictions

"A registrant would need permission to use protected data to read-across from a single substance to the target substance, ... But they would not need this to make a Qsar prediction."





**10,000 chemicals
800,000 tox
studies
(Dec 2014)**



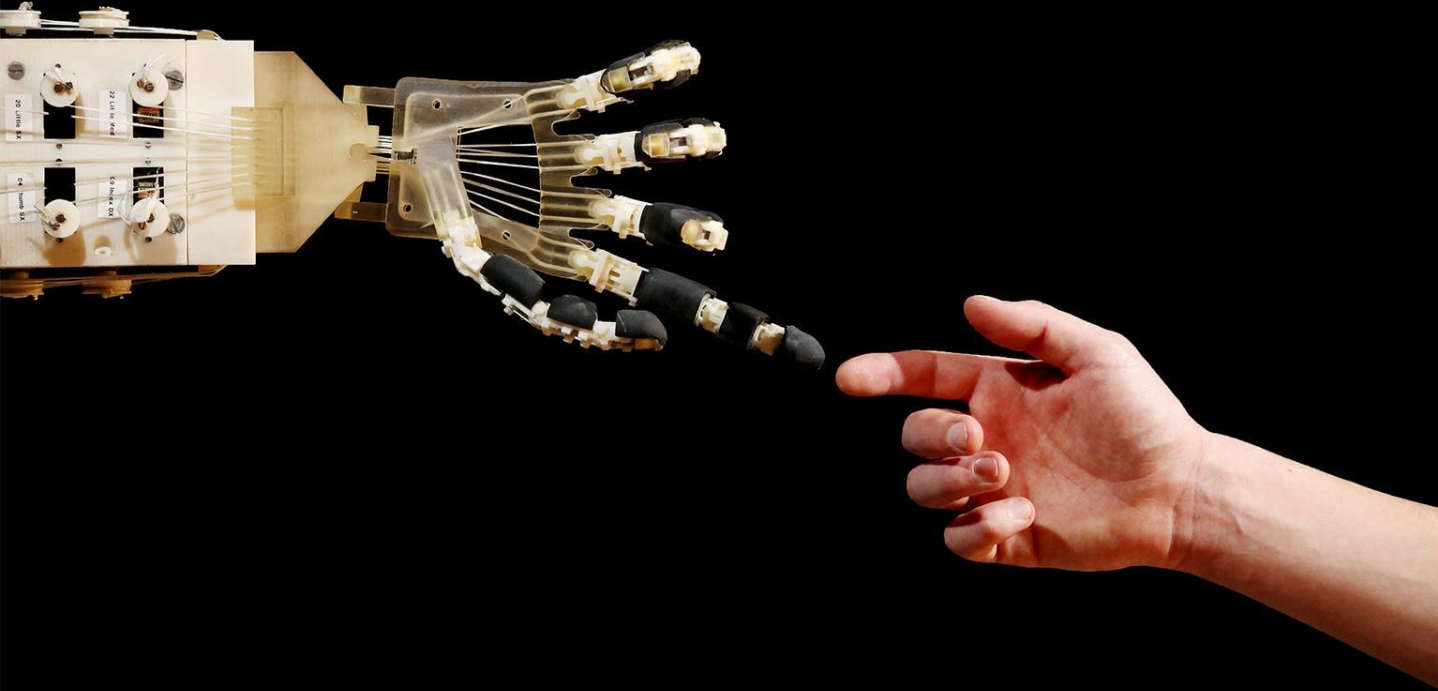
**Natural language
processing
(Feb 2016)
&
Web app**

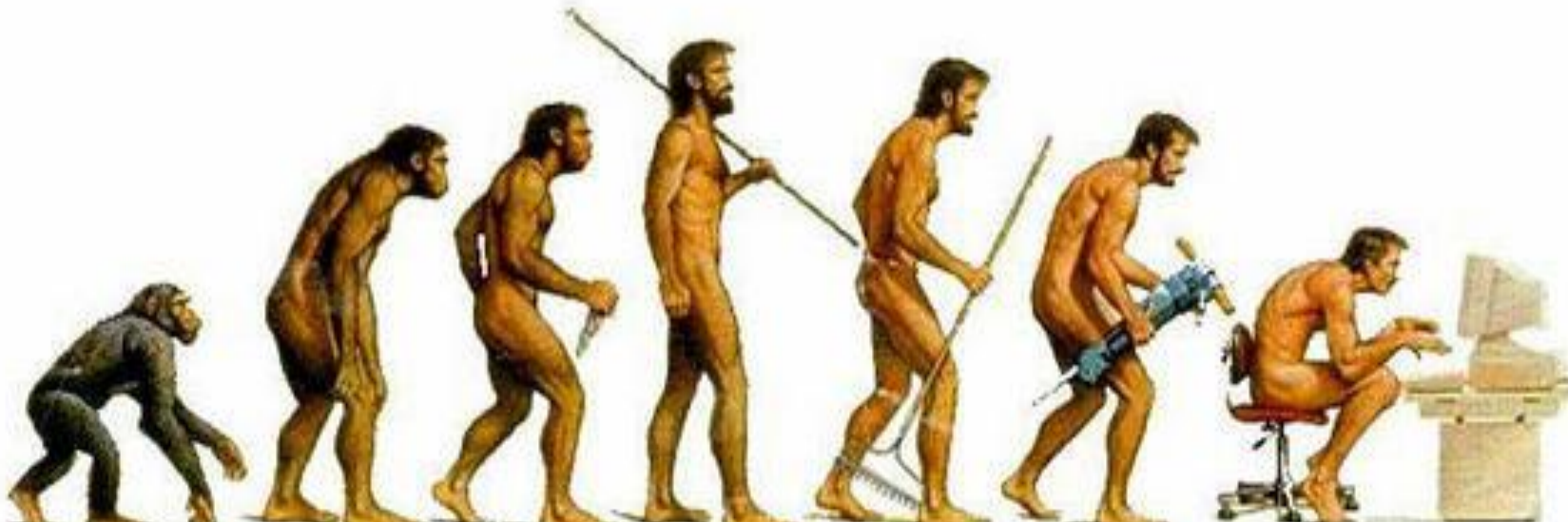
TOXTRACK



**10+ million
chemicals
300,000 with biol. &
20,000 with animal
data
(Mar 2017)**

A.I. is making big sense of big (complex) data





ALTEX 2017, 34:459-478

*“Big Data is like teenage sex:
everyone talks about it, nobody really knows
how to do it, everyone thinks
everyone else is doing it, so everyone
claims they are doing it.”*

Dan Ariely, Professor of Psychology
and Behavioral Economics at Duke University

Food for Thought ...

Computational Approaches to Chemical Hazard Assessment

Thomas Luechtefeld¹ and Thomas Hartung^{1,2}

RASAR - A marriage of technologies

Read-across

- Support weight of evidence
- Circumstantial
- Manual
- Unclear acceptability

(Q)SAR

- Data-mining by computer
- Broader applicability
- Can be validated with enormous consequences for acceptability

Read-Across-based Structure Activity Relationship = RASAR

- Mines local “similarity space”
- Comprehensive use of available data (data fusion)
- Expresses certainty
- Validation on the way



The map of the chemical universe

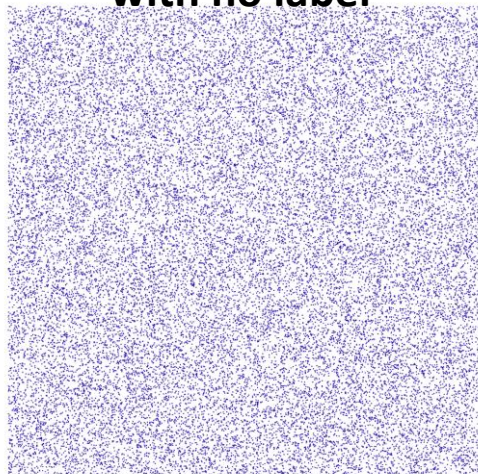
**Similarity =
proximity**

**ARTIFICIAL
INTELLIGENCE
0,5 BILLION
CALCULATIONS
PER PREDICTION**

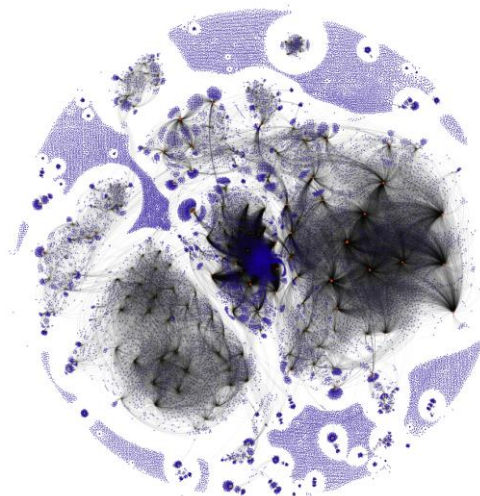


Modelling of sufficiently close neighbor availability with increasing number of chemicals with data

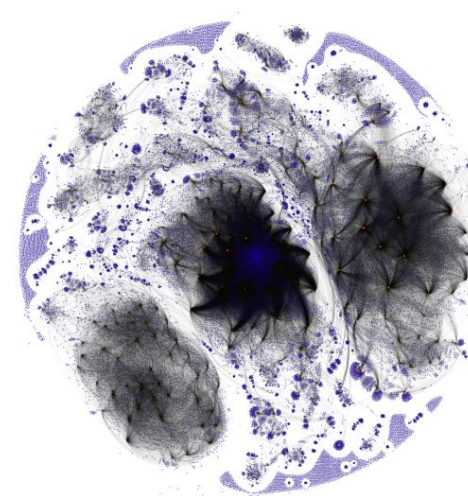
**33.383 chemicals
with no label**



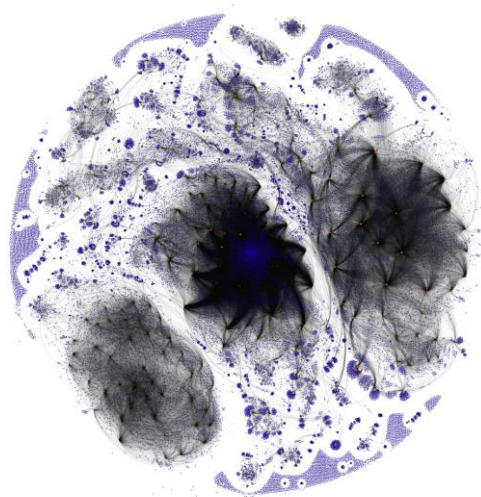
300 chemicals with label



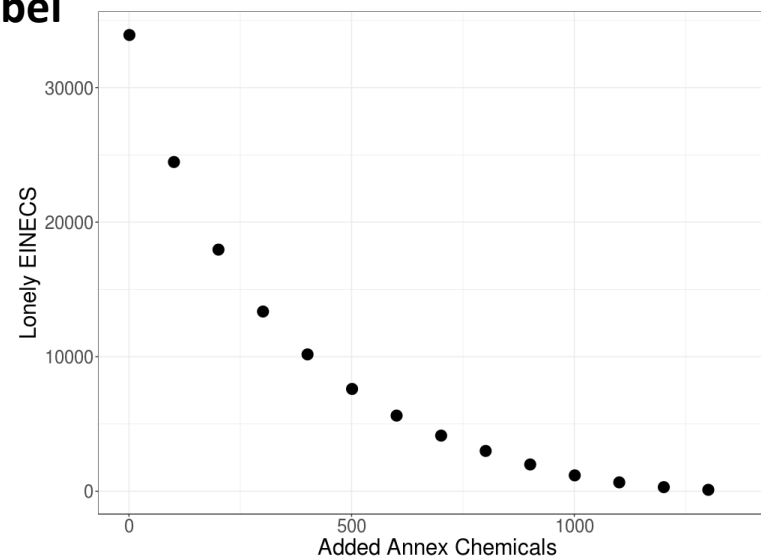
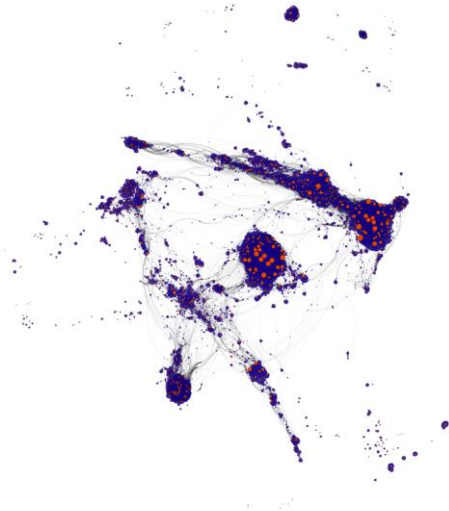
600 chemicals with label



900 chemicals with label



1,387 chemicals with label



REACHAcross

REACHAcross™ My Requests New Request

Welcome to REACHAcross™ software a reliable digital assistant for REACH compliance.

Offering the best of both worlds, REACHAcross™ software combines an objective computational approach of a QSAR with the proven accuracy of read-across systems. Generate REACH dossier compliant in minutes.

Need assistance?

[Contact us](#) for technical support.



REACHAcross™



REACHAcross™

Request Name REACHAcross™ Questions

Request Name ExampleChemicals Request ID 1401627

[View Summary](#)

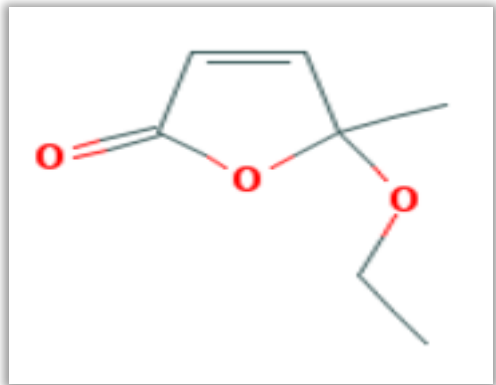
REACHAcross™ Questions

Chemical: (SMILES or CAS Registry Number. REACHAcross™ 1.0.0 does not currently use European Inventory of Existing Commercial chemical Substances (EINECS) numbers to identify chemical structures.)

Endpoint Selection:

<input checked="" type="checkbox"/>	Document	
<input checked="" type="checkbox"/>	Acute Dermal Irritation	
<input checked="" type="checkbox"/>	Acute Dermal Toxicity	
<input checked="" type="checkbox"/>	Acute Eye Irritation	
<input checked="" type="checkbox"/>	Acute Oral Toxicity	
<input checked="" type="checkbox"/>	Mutagenicity	
<input checked="" type="checkbox"/>	Skin Sensitization	

[Back](#) [Save and Next >](#) [Save & Exit](#)



REACHACROSS™ REPORT


REACHAcross™ Report

REACHAcross™ 1.0.0 estimates a 95% probability of Acute Oral Toxicity hazard for OC.

The below resources will aid in completing your IUCLID submission:

1. [ECHA - How to use and report Q/SARs](#)
2. [REACHAcross™ Documentation](#) <http://ulreachacross.com>
3. [REACHAcross™ QMSE](#) <http://ulreachacross.com/Documents/reachacross-1.0.0-qmrf.xml>
4. [REACHAcross™ QPRE](#) <http://ulreachacross.com/Documents/reachacross-1.0.0-qprf.txt>

The below information is supplied to aid in completing a(n) Acute Oral Toxicity submission in IUCLID:

ADMINISTRATIVE DATA

Type of information:
(Q)SAR

Reliability:
2 (reliable with restrictions)

Rationale for reliability:
Results derived from a valid (Q)SAR model and falling into its applicability domain, with adequate and reliable documentation / justification.

Justification for type of information

Software:
<http://ulreachacross.com/>

Model (incl. version number):
REACHAcross™ v1.0.0

SMILES or other identifiers used as input for the model:
OC

Scientific validity of the (Q)SAR model:

- Defined endpoint: Acute Oral Toxicity
- Unambiguous algorithm: REACHAcross™ provides an unambiguous algorithm definition at <http://ulreachacross.com/Documents/reachacross-1.0.0-wp.pdf>
- Defined domain of applicability: REACHAcross™ 1.0.0 defines a probabilistic domain of applicability. Substances predicted with sufficiently high or low probability are included in the domain of applicability.
- Appropriate measures of goodness-of-fit and robustness and predictivity: REACHAcross™ 1.0.0 uses leave one out cross validation on the ECHA C&L database. These results are reported at <http://ulreachacross.com/Documents/reachacross-1.0.0-wp.pdf>
- Mechanistic interpretation: N/A

Applicability domain:

- Descriptor domain: Pubchem2D Fingerprints and Similarity Network Features
- Structural and mechanistic domains: Defined by model predictions.

2 of 4
UL and the UL logo are trademarks of UL LLC ©

Example Predictions

REACHAcross 1.0.0 hazard estimates (x) for 12 REACH Annex compounds. Red bars show known hazards.

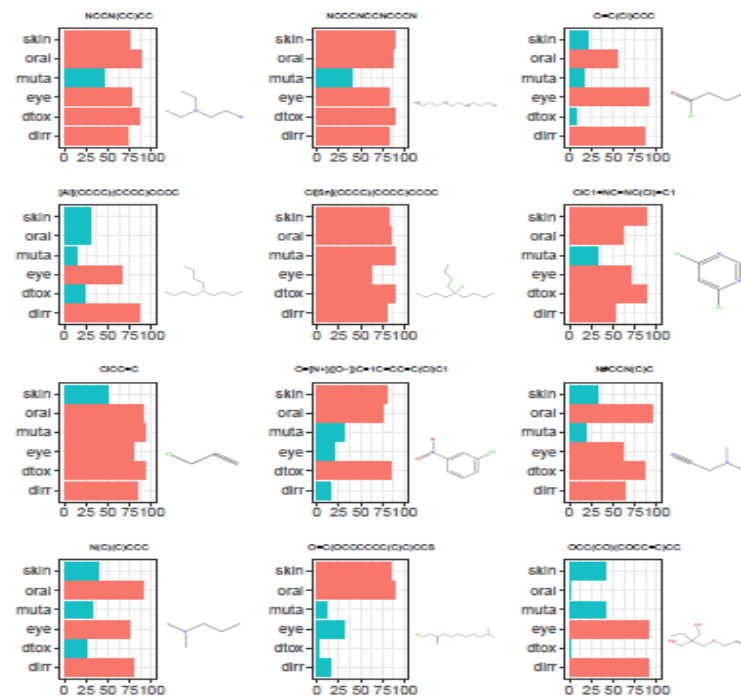


Figure 1: estimated hazard probability for 12 chemicals in annex 6 table 3.1



Table 1 Sensitivities (Se) and specificities (Sp) for 6 health hazard models built from thousands of classification and labelling results stored on the ECHA database

Endpoint	Tested	Se	Sp	Coverage
Skin sensitization	5136	83%	55%	83%
Eye Irritation	15 214	83%	54%	79%
Acute oral	12 342	82%	71%	77%
Mutagenicity	4077	80%	58%	81%
Skin irritation/corrosion	14 718	88%	57%	64%
Acute dermal	6732	89%	70%	59%

58,000 predictions, 42,500 possible



Toxicology Research

REVIEW

[View Article Online](#)

[View Journal](#)



Check for updates

Big-data and machine learning to revamp computational toxicology and its use in risk assessment

Cite this: DOI: 10.1039/c8tx00051d

Thomas Luechtefeld,^a Craig Rowlands^b and Thomas Hartung *^a

Toxicological Research 2018, in press, doi:10.1039/C8TX00051D

Available online

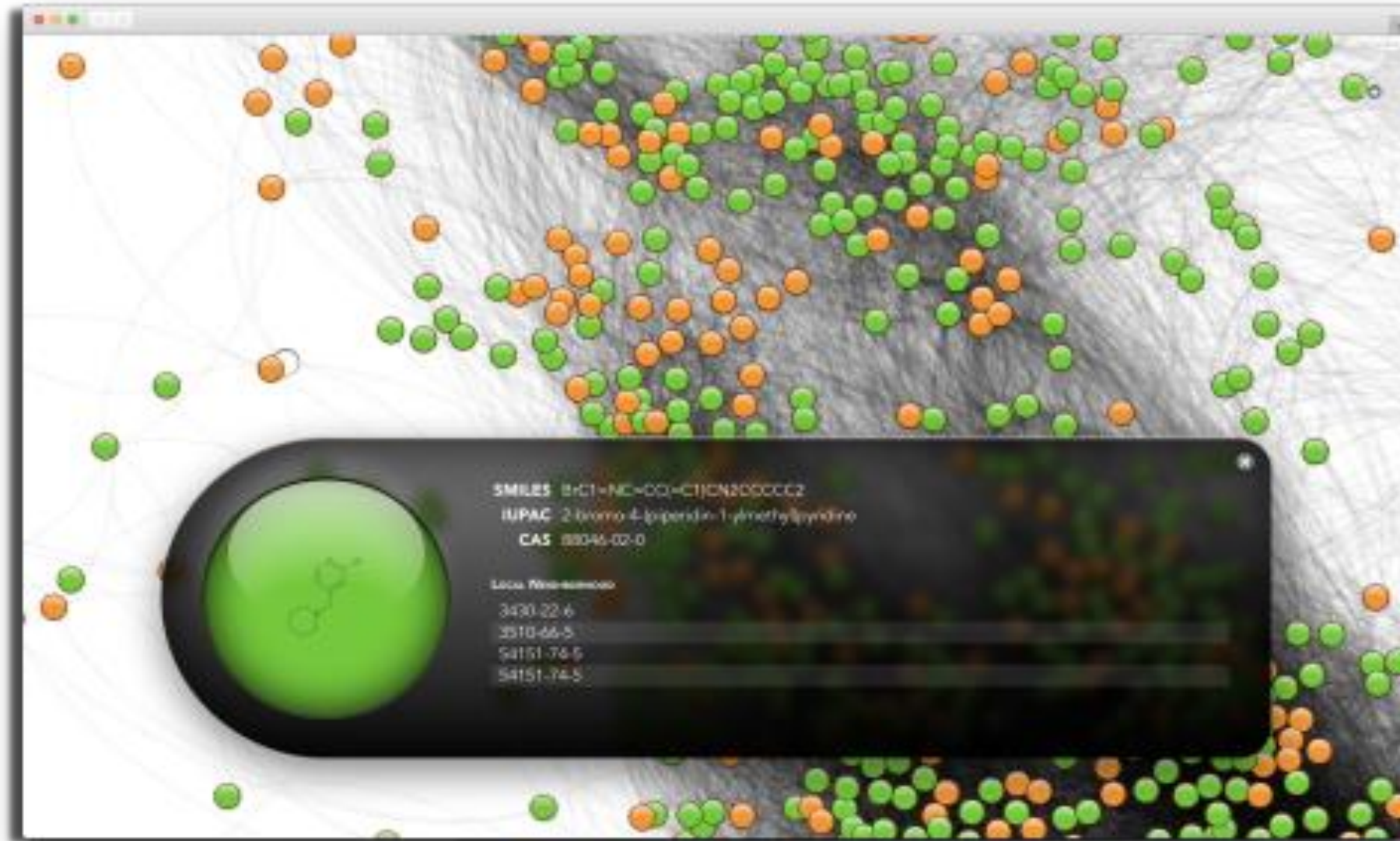
CHEMICAL UNIVERSE – 2018 DATABASE



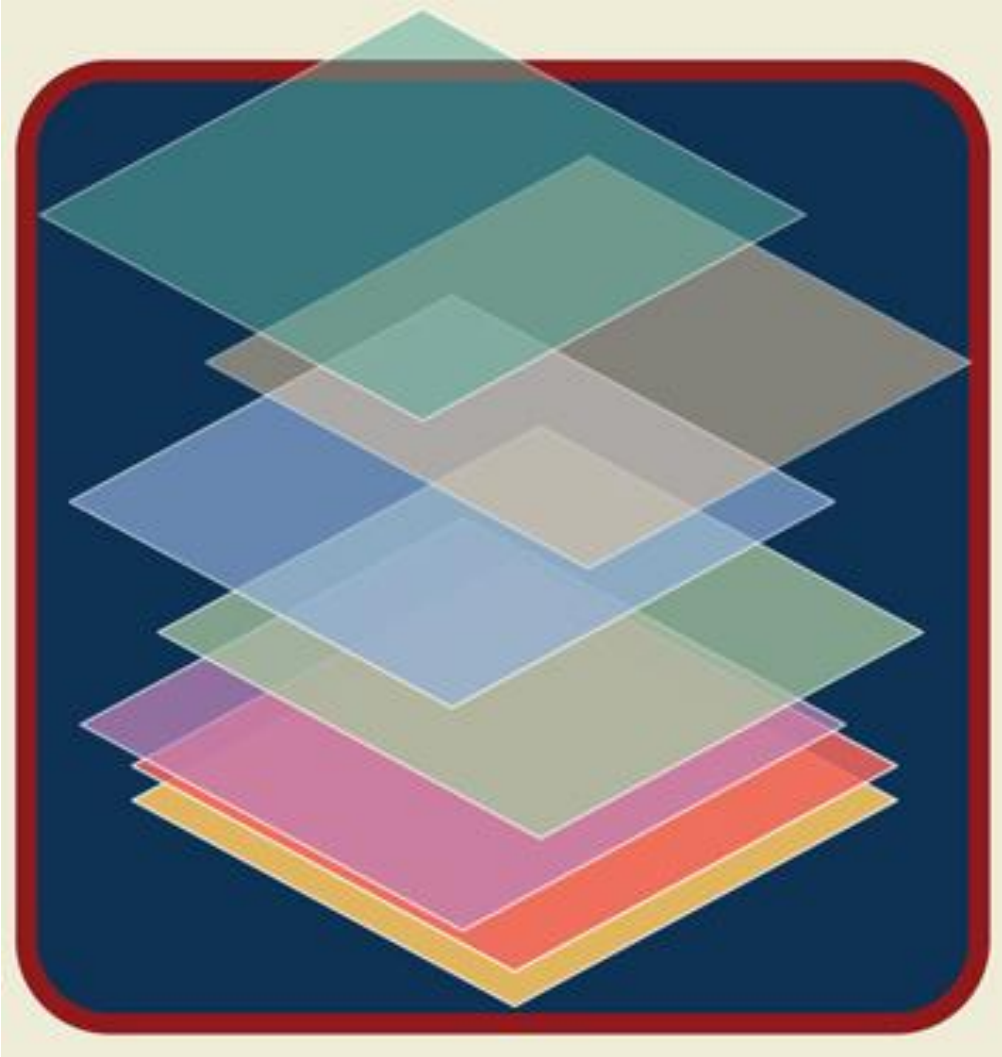
 **COLLABORATION**

10 million compounds
50 trillion comparisons

**2 days on Amazon cloud
server**



The next level: DATA FUSION



**Do not analyze
hazards
independently,
but let them
inform each
other**

Then next level: DATA FUSION

Hazard	Chemicals	Sensitivity	Specificity	BAC %	ACC %
Acute Aquatic Binary	10,541	95	94	95	95

190,000 predictions
87% correct

Skin Corrosion Binary	46,331	98	75	86	97
Skin <u>Sensitisation</u> Binary	7,670	80	96	88	84

Coverage 100% !

Published 11 July 2018

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



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International journal of science

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News Opinion Research Analysis Careers Books & Culture

NEWS • 11 JULY 2018

Software beats animal tests at predicting toxicity of chemicals

Machine learning on mountain of safety data improves automated assessments.

42

 5



An estimated 3 million to 4 million rabbits, rats, and other animals are used annually around the world for chemical safety tests. CAIRNEY DOWN/ALAMY STOCK PHOTO

New digital chemical screening tool could help eliminate animal testing

By Vanessa Zainzinger | Jul. 11, 2018, 11:00 AM

Six most used tox tests - 55% of animals in tox

Animal repeat test: 81% (balanced) accuracy

A.I. prediction: 87 % (balanced) accuracy

for 4-48.000 chemicals with animal data

2018 first regulatory acceptance of REACH*across* (Korea)

Luechtefeld et al., ToxSci 2018

Formal validation will have to show,

simple.

whether we can get information for the most used animal tests now by pressing a button?

working on it...



REACH*across*TM

- Different markets & industries

Cheminformatics Suite



- Validation
- Comparison with other tools
- Regulatory acceptance

TOXTRACK

- Engine 2.0
- Thresholds of Tox Concern



UL Cheminformatics Suite

Behind firewall

Combine proprietary data

Customized user interface

- **Run lists of chemicals**
- **Chemical design**
- **1-on-1 comparison for alternative chemistry**
- **Identify alternative chemicals**

*Finding alternative
Chemicals*

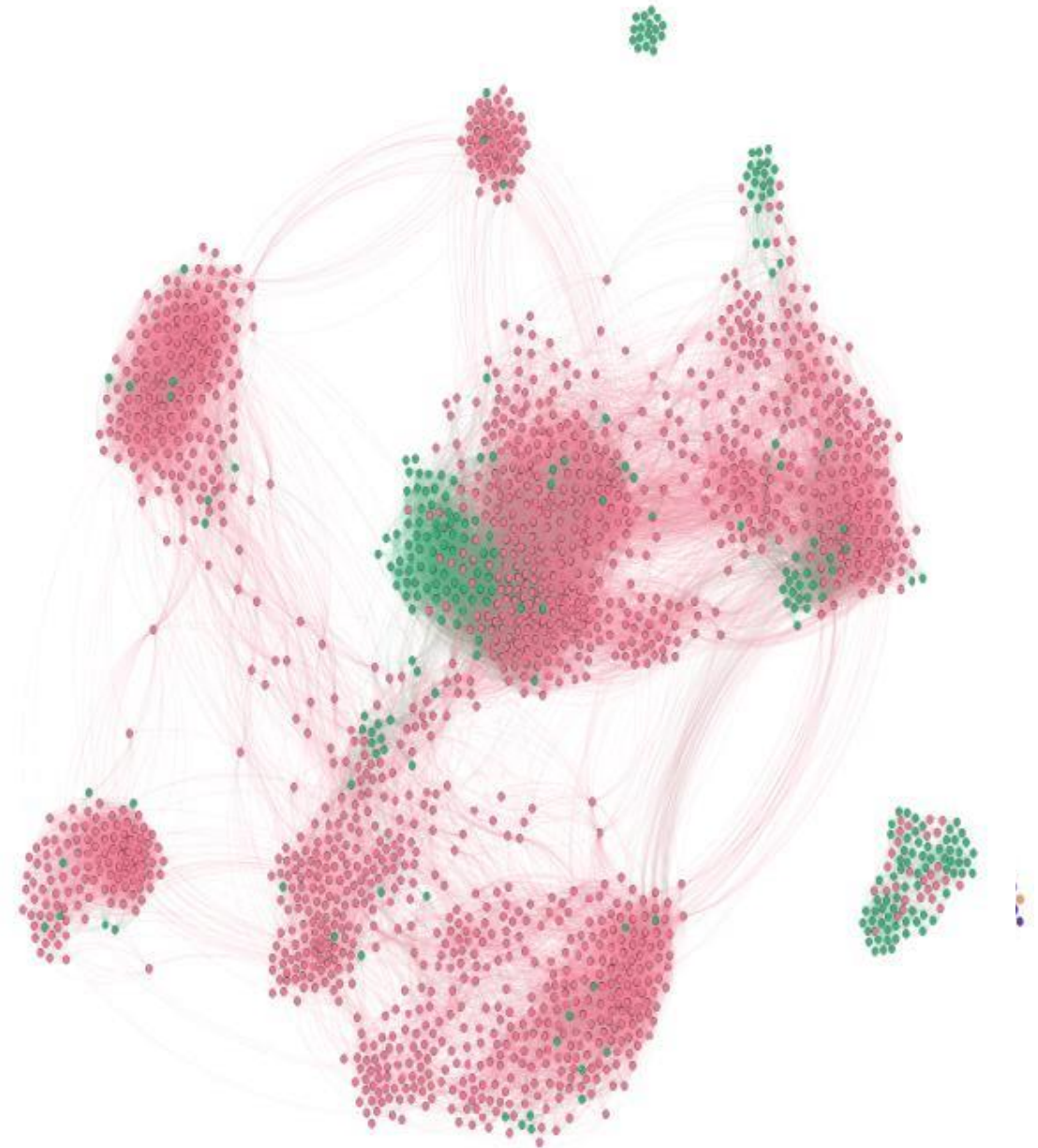
Example

Dichloromethane

1. Tox space

**2. Chemical
Similarity Space**

**3. Optimized
Combination**





Food for Thought ... Green Toxicology

*Alexandra Maertens¹, Nicholas Anastas³, Pamela J. Spencer⁴, Martin Stephens¹,
Alan Goldberg¹ and Thomas Hartung^{1,2}*

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



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



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

doi: 10.1093/toxsci/kfx243

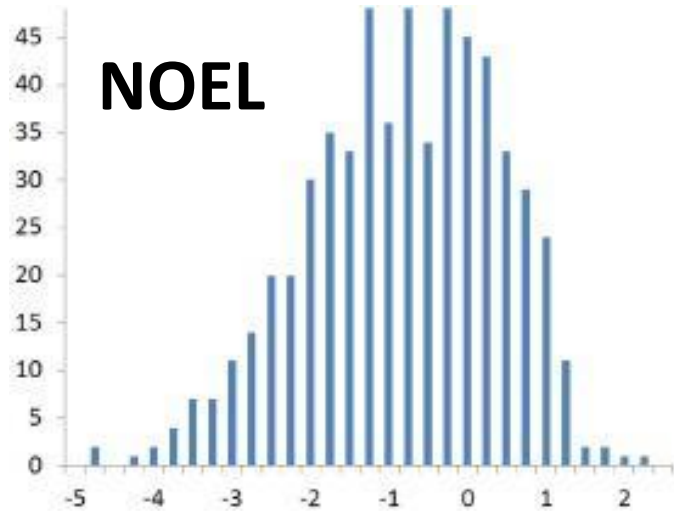
Advance Access Publication Date: December 18, 2017

Editorial

EDITORIAL

Green Toxicology—Know Early About and Avoid Toxic Product Liabilities

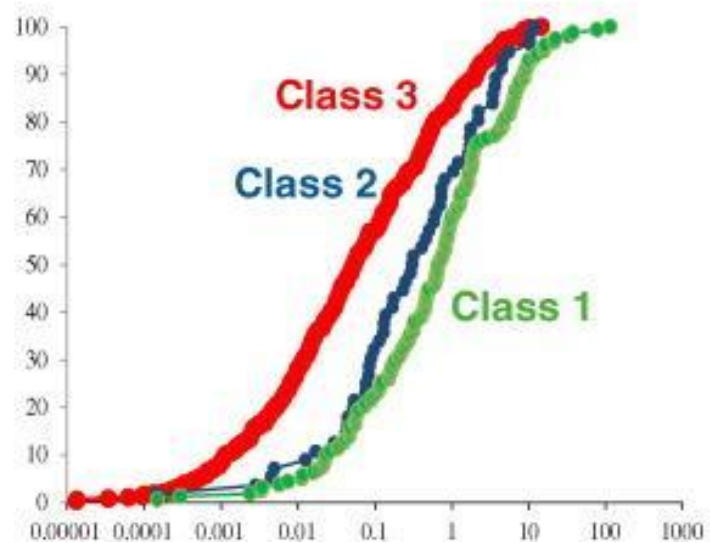
Alexandra Maertens* and Thomas Hartung*,†,1



Threshold of Toxicological Concern (TTC)

Concept:

- No untested substance will be much more toxic than all (similar) tested ones
- Compare to dose of use scenario



Very pragmatic de-risking

Food for Thought ...

Thresholds of Toxicological Concern – Setting a Threshold for Testing below Which There Is Little Concern

Thomas Hartung

ALTEX 2017,
34:331-351



Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

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



The Threshold of Toxicological Concern for prenatal developmental toxicity in rats and rabbits



B. van Ravenzwaay^{a,*}, X. Jiang^a, T. Luechtefeld^b, T. Hartung^{b,c}

No concern ← ↓ → Testing & Risk

Board Request May 2017

EFSA invitation June 2017

$$f(\text{trash}) = \text{trash}$$

Quality of animal data

Lack of (public) animal data

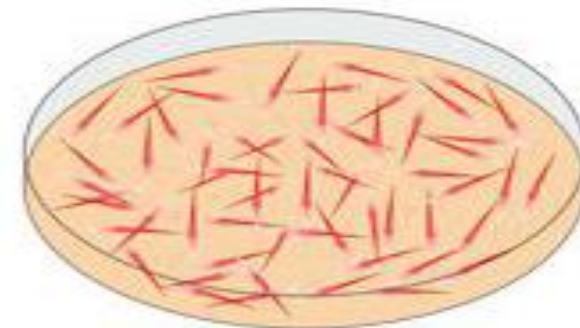
**Complex endpoints: chronic, cancer,
reproductive toxicity...**

We still need testing!



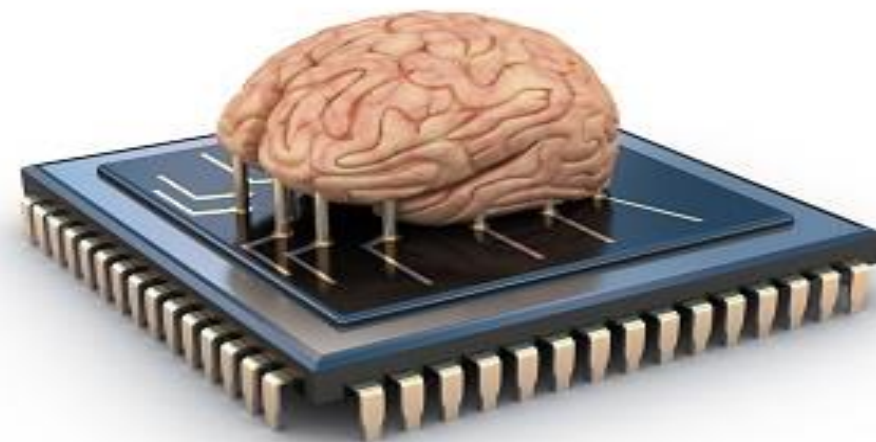
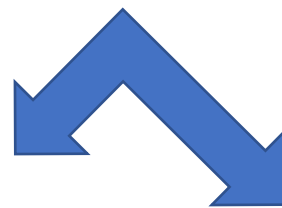


Gold Standard?

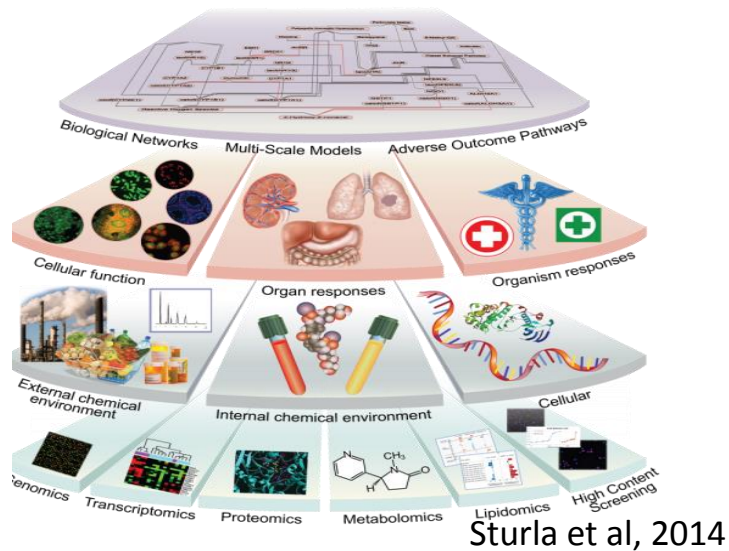


2D culture

GOAL



MPS



Systems
Toxicology



"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)

"Systems thinking is a discipline for seeing wholes. It is a framework for seeing interrelationships rather than things, for seeing 'patterns of change' rather than static 'snapshots'."

Peter M. Senge (1947-), MIT

Food for Thought ...

3S – Systematic, Systemic, and Systems Biology and Toxicology

Lena Smirnova¹, Nicole Kleinstreuer², Raffaella Corvi³, Andre Levchenko⁴, Suzanne C. Fitzpatrick⁵ and Thomas Hartung^{1,6}

Chem Res Toxicol 2017, 30:870–882

**Chemical
Research in
Toxicology**

Perspective

pubs.acs.org/crt

Systems Toxicology: Real World Applications and Opportunities

Thomas Hartung,^{†,‡,Ⓜ} Rex E. FitzGerald,[§] Paul Jennings,^{||} Gary R. Mirams,[⊥] Manuel C. Peitsch,[#] Amin Rostami-Hodjegan,^{∇,○} Imran Shah,[◆] Martin F. Wilks,[§] and Shana J. Sturla^{*,¶,Ⓜ}

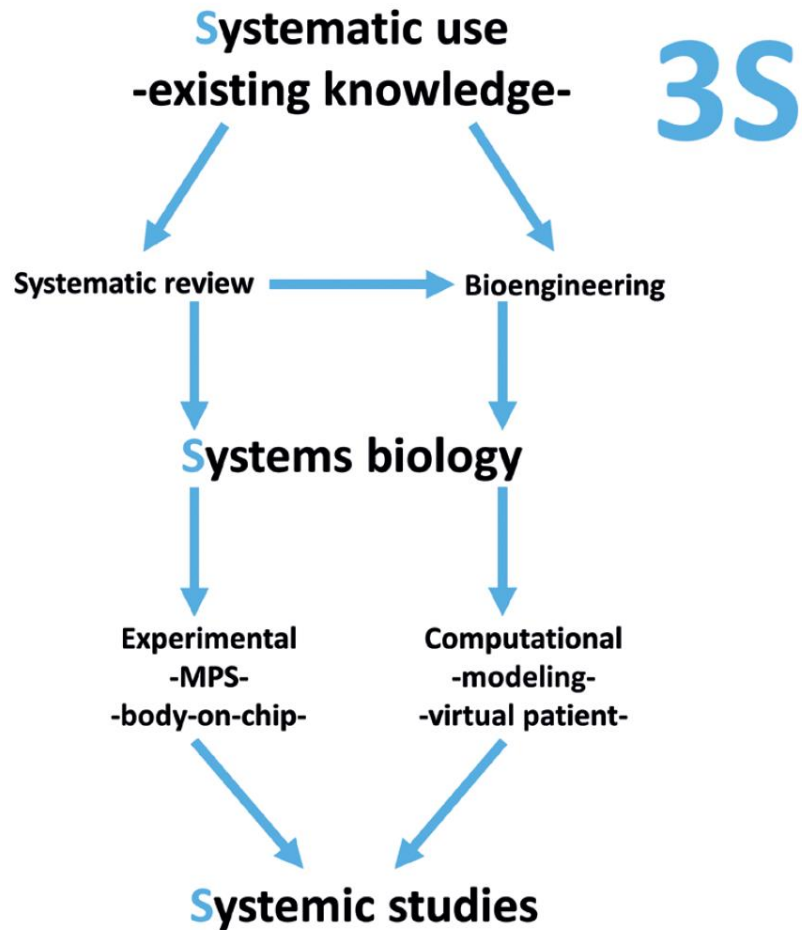


Fig. 1: The 3S approach to study systemic phenomena

Autism Spectrum Disorders



Data from Studies completed by the CDC on Autism Spectrum Disorder incidence rates.

EMERGING NEW DISEASES

**DEVELOPMENTAL
NEUROTOXICITY IS
THE ENDOCRINE
DISRUPTOR PROGRAM
OF THE NEXT DECADE**

2013: 1 in 68 children (CDC)

**2014: 1 in 59 children
(CDC 27 April 2018)**

**Animal test:
\$1,4 million**

1,400 animals

**200 chemicals tested:
No regulatory
consequence**

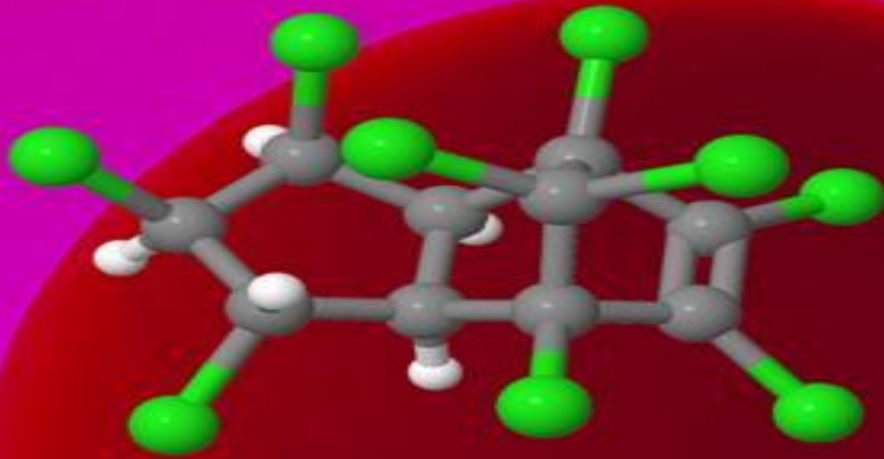


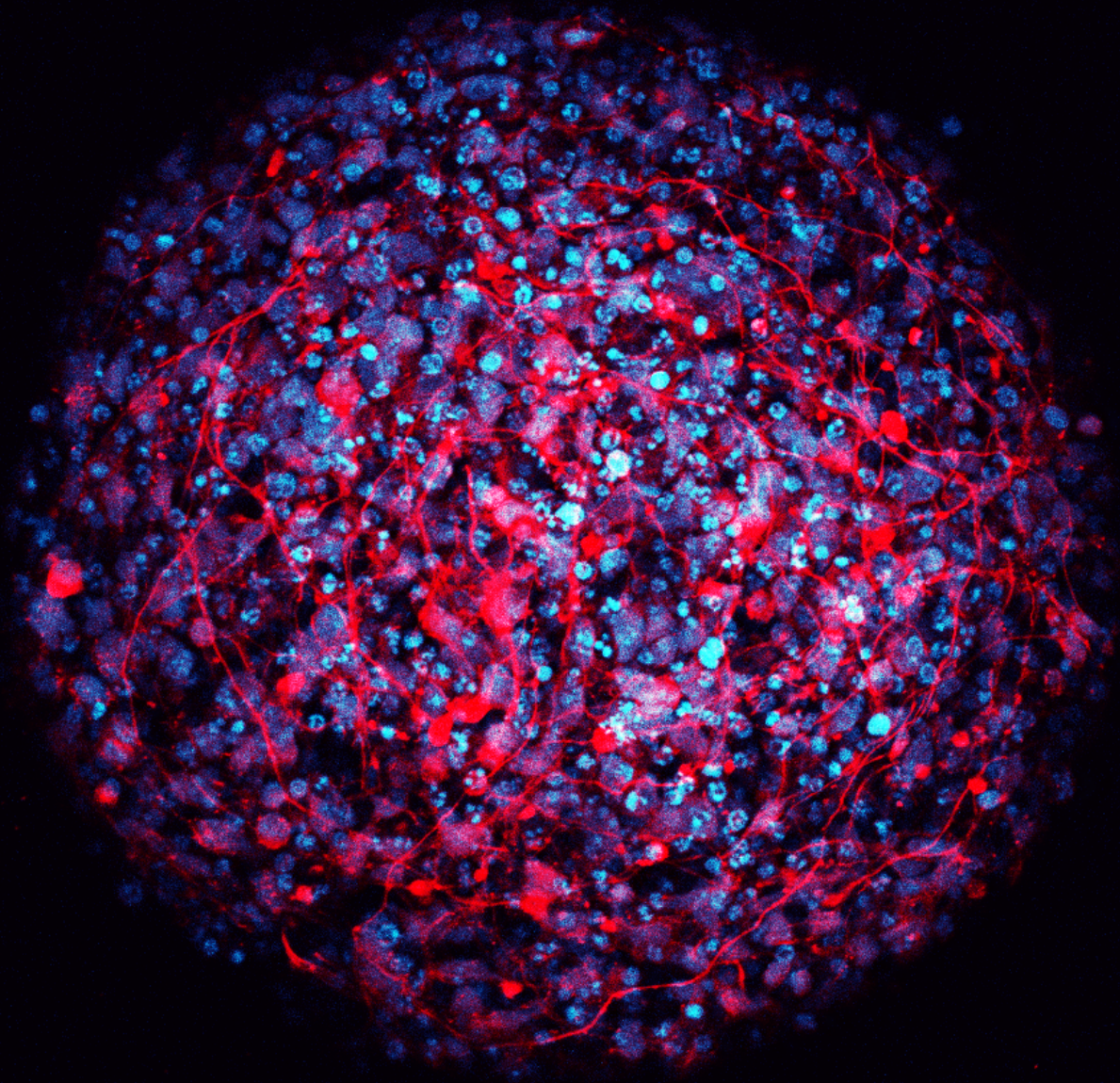
Food for Thought ...

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

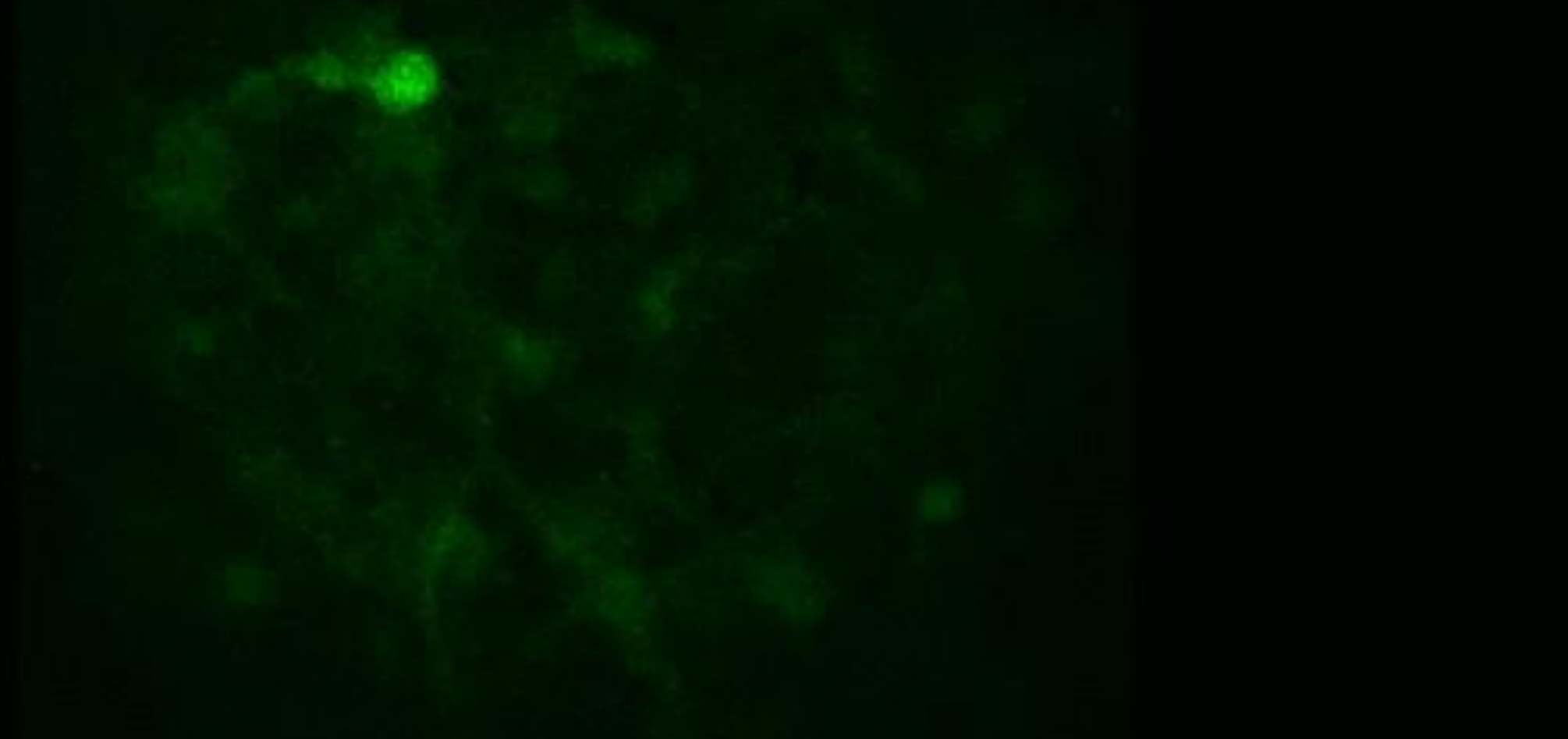




OUR MINI-BRAIN PROJECT

- **FROM SKIN OF DONORS,
INDIVIDUAL STEM CELLS**
- **IN 3 MONTHS
THOUSANDS OF
IDENTICAL ORGANIDS**
- **NEURONS
COMMUNICATING**
- **SOME BRAIN
FUNCTIONALITY**

Human mini-brains spontaneously electrophysiologically active



50 μm

Courtesy of: Dr. Tzahi Cohen-Karni
Carnegie Mellon University

DISEASES LIKE AUTISM CANNOT BE EXPLAINED BY GENETICS OR EXPOSURE ALONE



DISPOSITION TO TOXICANTS?



TEST IN MINI-BRAINS WITH GENETIC BACKGROUND THAT ALLOWS DEVELOP-MENTAL NEUROTOXICITY



MINI-BRAINS

FT Weekend

US edition

Life & Arts Rhythm and booze | Diary On the US campaign trail | Person in the News Beyoncé

USA \$100 Canada C\$50 SATURDAY 15 FEBRUARY / SUNDAY 16 FEBRUARY 2016

Syrian rebels warn over ceasefire plan

A woman and her children run for cover following air strikes by Syrian regime forces on the outskirts of the capital Damascus yesterday. Syria's official opposition welcomed a plan for a nationwide 'cessation of hostilities' in the five-year civil war but warned Washington and allies they must continue to develop back-up plans should the diplomatic effort fail.

The temporary end to fighting announced by the International Syria Support Group, the countries whose influence is critical to the outcome of the conflict, is intended to pave the way for a permanent ceasefire and new elections.

Syria photo by AP



Briefing

- Hollnath hits at Argentine debt offer**
One of Argentina's hedge fund investors has criticised the country's attempt to put an end to a financial blockade, which received a sympathetic hearing from a US judge this week. — PAGE 2
- Chomsky defends Bernanke over emissions**
Renowned chief economist Carlos Diez has launched a belated defence of the Fed's chairman in the face of criticism that it was being peddled into the European-debt recovery scandal. — PAGE 8
- Eurozone recovery remains sluggish**
The eurozone's recovery remained sluggish in the final three months of 2015, recording growth of 0.5 per cent for the second quarter in a row. — PAGE 3
- Abenomics under severe threat**
If this week's yen surge to ¥112 against the dollar continues, it will hit the exports, profits and confidence of Japanese business hard. — PAGE 2
- Party's over for oil-producing nations**
Those oil-producing nations used to flaunting champagne-soaked jashneries in the era of 2000 oil were notably restrained at London's International Petroleum Week, with beer and water white wine reflecting the new reality. — PAGE 11

Banks fight to regain confidence

European and US groups stage strong rally • Deutsche set for \$5.4bn bond buyback

Big banks battled to regain investor confidence yesterday after a tumultuous week in which many suffered double-digit share price falls amid doubts about their ability to weather weaker global growth and ultra low interest rates.

European lenders rallied across the board after Deutsche Bank led the fight back, announcing that its "strongly liquid" position "would allow it to buy back \$5.4bn of bonds."

Wall Street continued in a similar vein, boosted by an anonymous source last Thursday that Jamie Dimon, chief

executive of JP Morgan Chase, had spent \$2bn buying shares in his own bank, more than his 2015 pay package.

Spain's conservative PP government and Santander, the Spanish financial giant, have also sought to boost confidence in their banks, raising share prices by buying up stock in recent weeks.

"It is all over? Boy, I hope so, but I doubt it," said Jeff Harte, an analyst at JPMorgan in New York, who said that investors were suffering "a kind of psychological hangover" from the crisis that followed the collapse of Lehman Brothers in 2008.

Banks have been laid low all year through a combination of fears over falling oil prices, slowing growth, market turbulence in China and the fading power of central banks to support markets.

The pace of the sell-off picked up this week, as Janet Yellen, chair of the Fed and Bernanke, kept open the option of the US joining Japan and Europe in pushing interest rates below zero.

Additional reporting by James Heister in Frankfurt and Tokyo. Book to Market. FT Big Read page 5

Market commentary by Lee and Thomas

It's all over? Boy, I hope so, but I doubt it.

Jeff Harte, JPMorgan

US adults who used online dating

of population in each group

Age Group	2013 (%)	2015 (%)
18-24	12	20
25-34	15	25
35-44	10	18
45-54	8	15
55-64	5	10
65+	3	5
Total	10	18

Source: Pew Research

Corby

Trump's rise galvanises populists in heart of UK

How GB voters have been misled by Donald Trump's 'No Wall' speech, he said, days after the property mogul's triumph at New Hampshire.

Yet Mr Gibbins is not a resident of New Hampshire. She lives in Corby in Northamptonshire, England, where she is a holdover in the movement to pull the UK out of the EU. That campaign is heating up, with the green movement expected to announce an 'in-out' referendum any day now.

Teasing Trump's rocket fuel — PAGE 2

Mass-produced mini-brains to spark rethink over drug testing on animals

Human mini-brains, made from the neurons of a full-sized brain, will be mass-produced to replace animals in drug testing, a move that is likely to transform pharmaceutical research and development.

Researchers at Johns Hopkins University, Baltimore, have created using brain from human stem cells that grow into little balls of neurons about the size of a fly's eye.

The mini-brains, which fire electrical impulses and communicate via their neural networks, show the electrical activity characteristic of thinking, characteristic of thinking, characteristic of thinking and project leader, called this "a primitive type of thinking," stressing that because there was no "input or output", electrical activity was "meaningless" ... but the neurons are trying to communicate with each other."

He said 95 per cent of drugs that look promising in animal tests fail when transferred to humans. "We have rodent models have been useful, we are not 100 percent. And even though we are not built like either, you are not getting much better information from these balls of cells than from rodents."

Other research teams, including scientists at the Institute of Molecular Biotechnology in Vienna and Tufts University in the US, have produced larger mini-brains. The advantage of the Johns Hopkins system, which was presented to the American Association for the Advancement of Science yesterday, is that hundreds of identical mini-brains can be produced in a batch.

"We don't have the first brain model we are so claiming to have the best one but this is the most standardized one."

Fred Hartung said. "When testing drugs it is imperative that the cells being studied are as similar as possible to ensure the most comparable and accurate results."

Cells from patients with genetic traits or diseases — including Alzheimer's, Parkinson's and even autism — can be used to create mini-brains for R&D.

"Take autism," Fred Hartung said. "We know that neither genetics nor exposure to chemicals alone leads to the disease. Perhaps we can finally start our work with mini-brains from the skin of autistic children. They bring the genetic background, the researchers bring the chemicals in."

He added: "Nobody should have an excuse to still use the old animal models."

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Mass-produced mini-brains to spark rethink over drug testing on animals

NEURODEGENERATION: PARKINSON, ALZHEIMER, ALS... MAKE MINI-BRAINS FROM PATIENTS!

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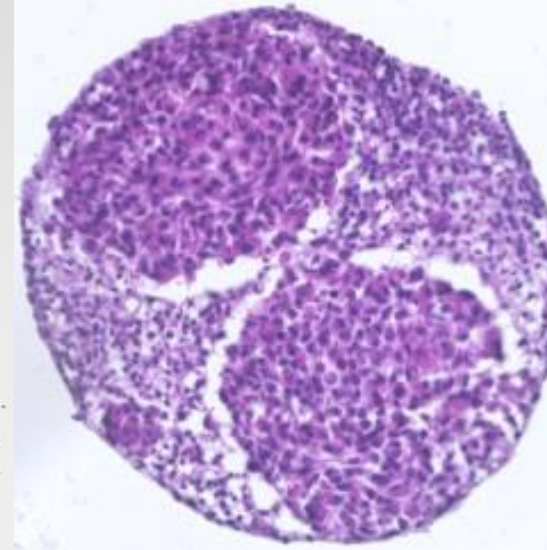
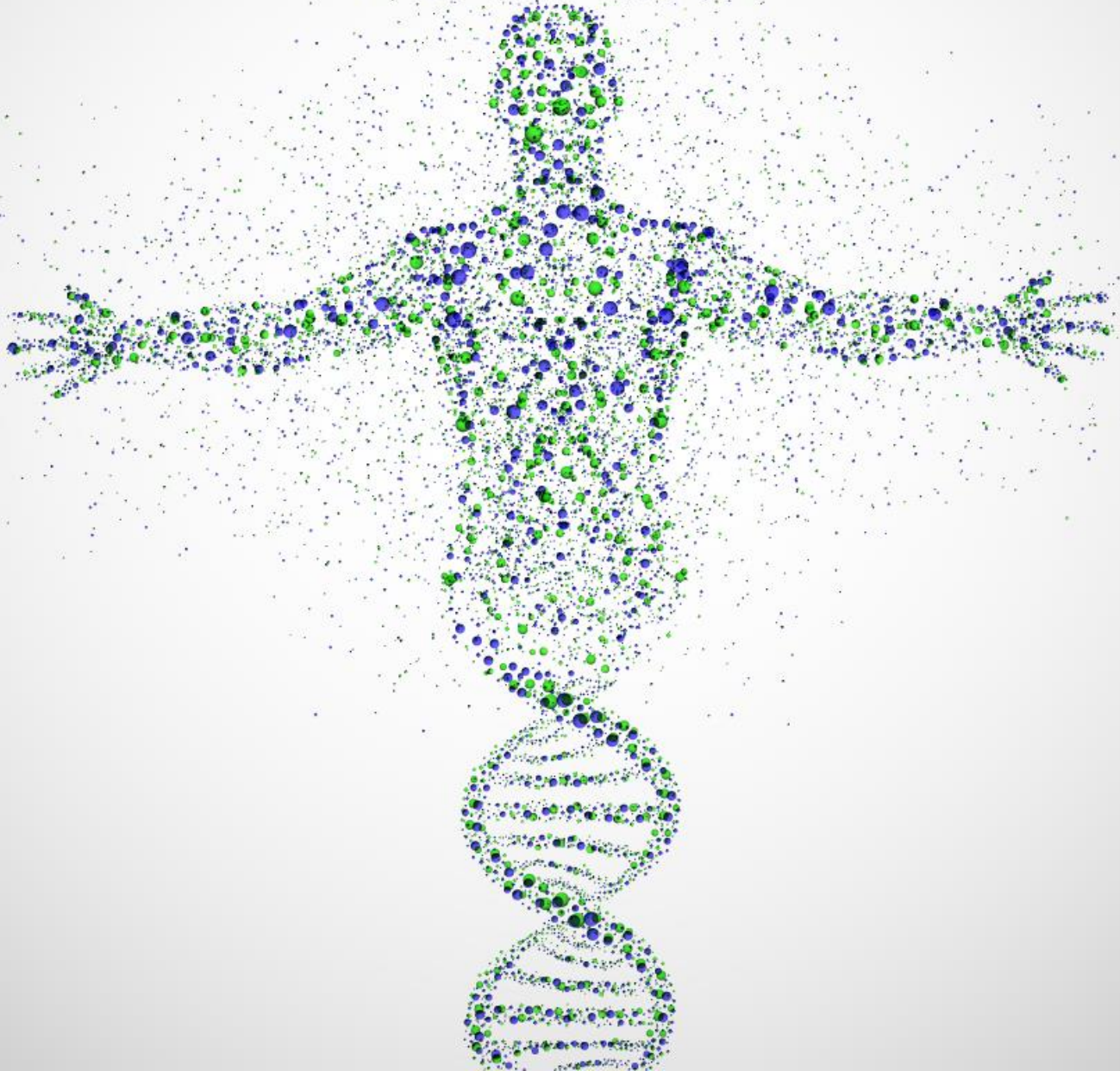
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Max 500MB

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STOCK MARKETS				COMMODITIES				INTEREST RATES			
MARKET	INDEX	CHG	%	COMMODITY	PRICE	CHG	%	COUNTRY	RATE	CHG	%
FTSE 100	6400.00	+100.00	+1.6	Oil (WTI)	48.50	-0.50	-1.0	US	0.25	0.00	0.0
NASDAQ	4100.00	+50.00	+1.2	Gold	1200.00	+10.00	+0.8	UK	0.50	0.00	0.0
DAX	12000.00	+150.00	+1.3	Natural Gas	3.50	-0.10	-2.8	EU	1.00	0.00	0.0
HANG SENG	23000.00	+300.00	+1.3	Copper	3.50	+0.05	+1.4	JP	0.00	0.00	0.0
NIKKEI 225	20000.00	+200.00	+1.0	Iron Ore	60.00	+1.00	+1.7	AU	4.00	0.00	0.0
ASX 200	7000.00	+100.00	+1.4	Platinum	1800.00	+20.00	+1.1	CA	1.00	0.00	0.0
IBEX 35	10000.00	+150.00	+1.5	Palladium	1500.00	+15.00	+1.0	IN	8.00	0.00	0.0
SEMI-CONDUCTORS	15000.00	+200.00	+1.3	Silver	20.00	+0.20	+1.0	Brazil	11.00	0.00	0.0
TELECOM	10000.00	+100.00	+1.0	Lead	2.00	+0.02	+1.0	India	9.00	0.00	0.0
ENERGY	12000.00	+150.00	+1.2	Zinc	1.50	+0.01	+0.7	China	6.00	0.00	0.0
FINANCIALS	11000.00	+100.00	+0.9	Aluminum	1.80	+0.01	+0.6	South Africa	9.00	0.00	0.0
RETAIL	10000.00	+100.00	+1.0	Nickel	1.50	+0.01	+0.7	South Korea	3.00	0.00	0.0
TECH	12000.00	+150.00	+1.2	Cobalt	1.50	+0.01	+0.7	Indonesia	8.00	0.00	0.0
HEALTHCARE	11000.00	+100.00	+0.9	Vanadium	1.50	+0.01	+0.7	Other	5.00	0.00	0.0
UTILITIES	10000.00	+100.00	+1.0	Uranium	25.00	+0.50	+2.0				

PERSONALIZED MEDICINE



JOHN McCAIN
BRAIN CANCER



GLIOBLASTOMA IN MINI-BRAIN

- **DEVELOP DRUGS**
- **OPTIMIZE CHOICE OF DRUG**

t4 Workshop Report*

Advanced Good Cell Culture Practice for Human Primary, Stem Cell-Derived and Organoid Models as well as Microphysiological Systems

David Pamies¹, Anna Bal-Price², Christophe Chesné³, Sandra Coecke², Andras Dinnyes^{4,5}, Chantra Eskes⁶, Regina Grillari^{7,8}, Gerhard Gstraunthaler⁹, Thomas Hartung^{1,10}, Paul Jennings¹¹, Marcel Leist^{10,12}, Ulrich Martin¹³, Robert Passier^{14,15}, Jens C. Schwamborn¹⁶, Glyn N. Stacey¹⁷, Heidrun Ellinger-Ziegelbauer¹⁸ and Mardas Daneshian¹⁰

**CAAT-EU: Reporting standards
Drafting of GCCP 2.0 has started**



Center for Alternatives
to Animal Testing



CAATwalk

March 5, 2018

News and Updates from CAAT

Call for Expression of Interest:
P4M—Public Private Partnership for
Performance Standards for
Microphysiological Systems



Education

Communication

Workshops

Strategic plans

Policy programs

Evidence-based approaches

Food for Thought ...

The Need for Strategic Development of Safety Sciences

Francois Busquet¹ and Thomas Hartung^{1,2}

¹Center for Alternatives to Animal Testing, CAAT-Europe, University of Konstanz, Konstanz, Germany; ²Johns Hopkins Bloomberg School of Public Health, Center for Alternatives to Animal Testing, Baltimore, MD, USA

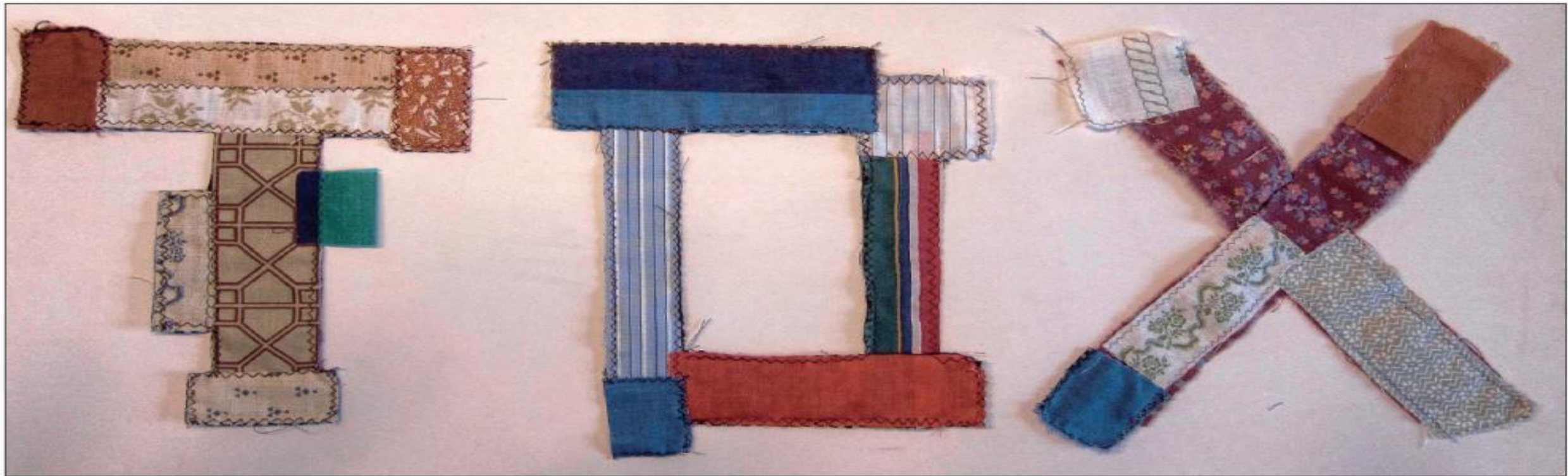


Fig. 1: The patchwork building of toxicology
(courtesy of Ingrid Hartung, Solingen, Germany)

Evidence-based toxicology!

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

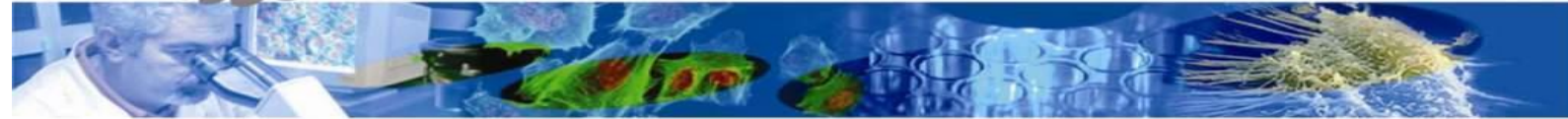
2006 Article

2007 Conference



EBT.

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



2009 Chair Hopkins

2011 Organization
www.ebtox.org

ebotc
Evidence-based Toxicology Collaboration

**I HAVE NEVER
HEARD OF AN**

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



Arch Toxicol
DOI 10.1007/s00204-017-1980-3

REVIEW ARTICLE

A primer on systematic reviews in toxicology

Sebastian Hoffmann^{1,13} · Rob B. M. de Vries² · Martin L. Stephens¹ · Nancy B. Beck³ · Hubert A. A. M. Dirven⁴ · John R. Fowle III⁵ · Julie E. Goodman⁶ · Thomas Hartung⁷ · Ian Kimber⁸ · Manoj M. Lalu⁹ · Kristina Thayer¹⁰ · Paul Whaley¹¹ · Daniele Wikoff¹² · Katya Tsaïoun¹ 

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,*}

Evidence-Based Toxicology Collaboration

I HAVE NEVER
HEARD OF AN




'UNSYSTEMATIC' REVIEW

imgflip.com

Arch Toxicol
DOI 10.1007/s00204-017-1980-3

REVIEW ARTICLE

A primer on systematic reviews in toxicology

Sebastian Hoffmann^{1,13} · Rob B. M. de Vries² · Martin L. Stephens¹ · Nancy B. Beck³ · Hubert A. A. M. Dirven⁴ · John R. Fowle III⁵ · Julie E. Goodman⁶ · Thomas Hartung⁷ · Ian Kimber⁸ · Manoj M. Lalu⁹ · Kristina Thayer¹⁰ · Paul Whaley¹¹ · Daniele Wikoff¹² · Katya Tsaïoun¹ 

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,i} Jennifer McPartland,^j Andrew A. Rooney,^k Roberta W. Scherer,^e Didier Verloo,^l and Sebastian Hoffmann^m

Environment International 92–93 (2016) 630–646



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,*}



CrossMark

Emerging EBT
concepts

EFSA – EBTC Colloquium, Lisbon 2017

The science of combining apples and oranges: Joint EFSA/EBTC scientific colloquium on evidence integration in risk assessment

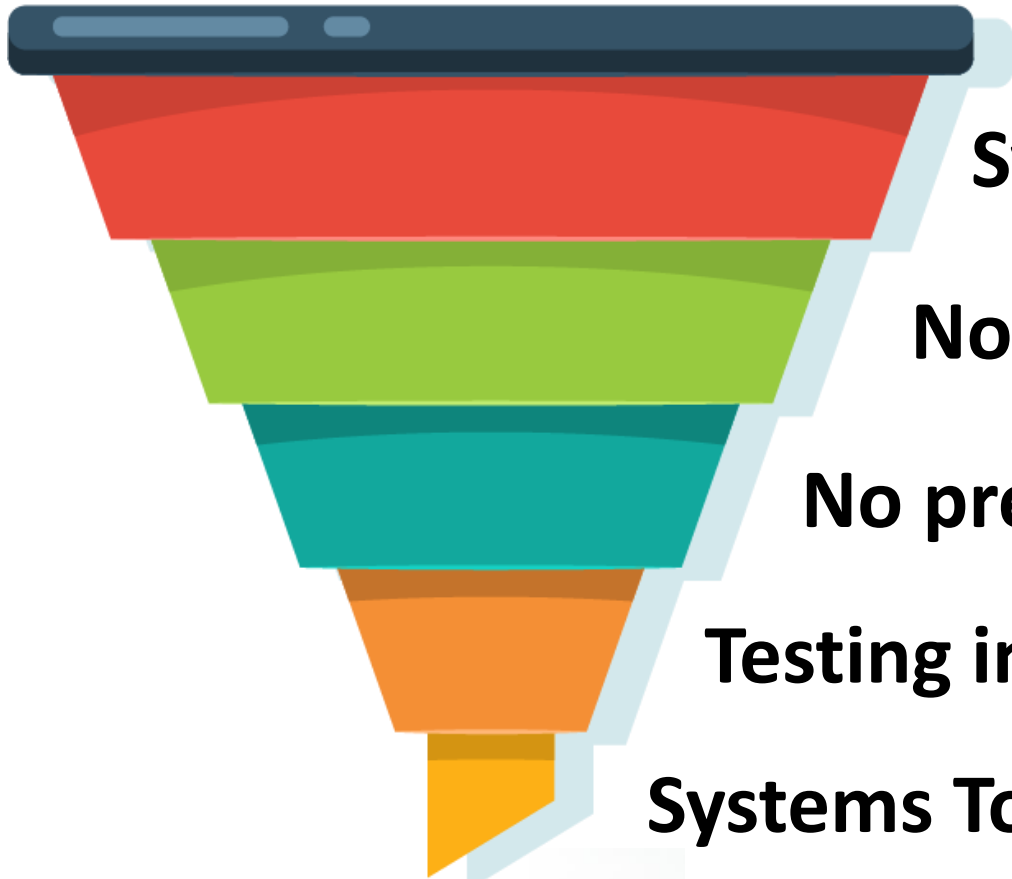
Lisbon, 25 October 2017

Report published: <https://www.efsa.europa.eu/en/supporting/pub/1396e>

1/5 Integrating Evidence For Hazard Identification (HI) (Part 1)



Our vision



Systematic review existing data (EBT)

No relevant exposure (TTC)

No prediction of hazard (A.I.)

Testing in meaningful cell models (MPS)

Systems Toxicology

Last resort



The need for change and EBT

Our strategy papers,
Workshop reports...



Tox-21c and EBT finally
promise implementation:

New technologies and a
framework for implementing
them by handling evidence
appropriately.

The New Yorker,
1'2017

(Online) courses at JHU: current and future

- **Tox21c Scientific Application** (L. Smirnova, T. Hartung)
- **Evidence-based Toxicology** (L. Smirnova, T. Hartung)
- **Humane Experimental Techniques and Animal in Law** (K. Hermann)
- **Green Toxicology** (Alex Maertens)
- **Computational Tools for Environmental Health** (A. Maertens)
- **Bioinformatics for Environmental Health** (A. Maertens)
- **Practical Ethics in Biomedical Sciences** (T. Hartung, L. Smirnova)
- **Reproductive and Developmental Toxicology** (L. Smirnova)



EDUCATION

Highlight Coursera

The screenshot shows the Coursera course page for "Toxicology 21: Scientific Applications". The page includes a navigation menu with options like Overview, Syllabus, FAQs, and Creators. A prominent "Enroll" button indicates the course starts on March 26. The course is created by Johns Hopkins University and is taught by Lena Smirnova. On the right, a "Weekly Course Digest" section displays learner statistics: Total Learners (634, up 26 from last week) and Active Learners (All Time) (364, up 15 from last week).

Weekly Course Digest

Toxicology 21: Scientific Applications

DASHBOARD

Total Learners ▲ 26
634
from last week

Active Learners (All Time) ▲ 15
364
from last week

About this course: This course familiarizes students with the novel concepts being used to revamp regulatory toxicology in response to a breakthrough National Research Council Report "Toxicity Testing in the 21st Century: A Vision and a Strategy." We present the latest developments in the toxicology field, moving away from animal testing toward human relevant, high content, high throughput integrative testing.

Created by: Johns Hopkins University

Taught by: Lena Smirnova, Research Associate
Center for Alternatives to Animal Testing

Released 2'18, EBT to follow 9'18

20th International Congress on *In Vitro* Toxicology (ESTIV2018)

New approach methodologies for in vitro toxicology applications

15-18 October 2018 • Berlin / Germany

CONGRESS DETAILS



- The European Society of Toxicology *In Vitro* (ESTIV)

- Gesellschaft für Toxikologie (GT, German Toxicology Society)

- Center for Alternatives to Animal Testing – Europe (CAAT-EU)



Prof. Mathieu
Vinken
ESTIV



Dr. Robert
Landsiedel
GT



Prof. Thomas
Hartung
CAAT-Europe

SAVE THE DATE



11th Congress on Alternatives and Animal Use in the Life Sciences

3Rs in transition

From development to application

23-27 August 2020

MECC Maastricht - The Netherlands



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

John Maynard Keynes

(1883 - 1946)

