Read-across initiatives to replace animal testing

Thomas Hartung & team

Center for Alternatives to Animal Testing

JOHNS HOPKINS
BLOOMBERG SCHOOL
OF PUBLIC HEALTH

1981 2010 2012






Stakeholder Platform

Transatlantic Hub

CAAT EU Policy Program

3Rs

Thanking our sponsors (industry, philanthropy, agencies)

Current

Space for You!

Recent

…and individuals

EUROPEAN COMMISSION

Conflict of Interest Statement

Founder (organoids)

Consultant

Consultant Computational Toxicology

Licensed Pyrogen Test Consultant

The problem:

Complex mixture

Many unknown

Variable composition

UVCB

Mass-spectrum of diesel fuel

And a lot of it…

ECHA

REACH 2018

Individual substances

Test needs by tonnage

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

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

ALTEX 2018, 35:275-305

REACH

Originally expected:

- **180.000 pre-registrations by about 27.000 companies**
- **30.000 substances**

State of the play 12’ 08:

- **> 2.7 million pre-**

nature

Vol 460|27 August 2009

OPINION

Chemical regulators have overreached

The costs — both in animal lives and euros — of the European REACH legislation on chemical testing are escalating. **Thomas Hartung** and **Costanza Rovida** argue for a suspension of certain toxicity tests.

How are we doing? - animal numbers

Substances by registration deadline (cumulative)	2010	2013	2018	Animals used
Commission estimate (2003)	2,704	5,165	29,324	4 million *(van der Jagt, 2002)* 8 -13 million *(ECVAM best –worst case, 2006)*
Actual	**4, 599**	**8, 729**	*49,851*	*If we get 70% as many registrations in 2018 we could* looking *at least 6 million* imals

+70% +70%

Total: 13,328

Our prediction: 12,007

Cruelty Free INTERNATIONAL

Slide from Katy Taylor, 2015
EU Coalition to End Animal Exp.

t4 Report*

ALTEX 2011, 28:273-294

How are Reproductive Toxicity and Developmental Toxicity Addressed in REACH Dossiers?

Costanza Rovida [1], *Fabiola Longo* [2], *and Richard R. Rabbit* [1]

[1] Center for Alternatives to Animal Testing (CAAT)-Europe, University of Konstanz, Germany; [2] Private Consultant, Malnate, Varese, Italy

The number of animals used … first REACH deadline, is very high; it may add up to 1.6 million animals just to accomplish reproductive and developmental toxicity endpoints if the data collected from 400 dossiers are extrapolated to the total number of registered substances.

40% existing data (but quality concerns), only 11% propose tests

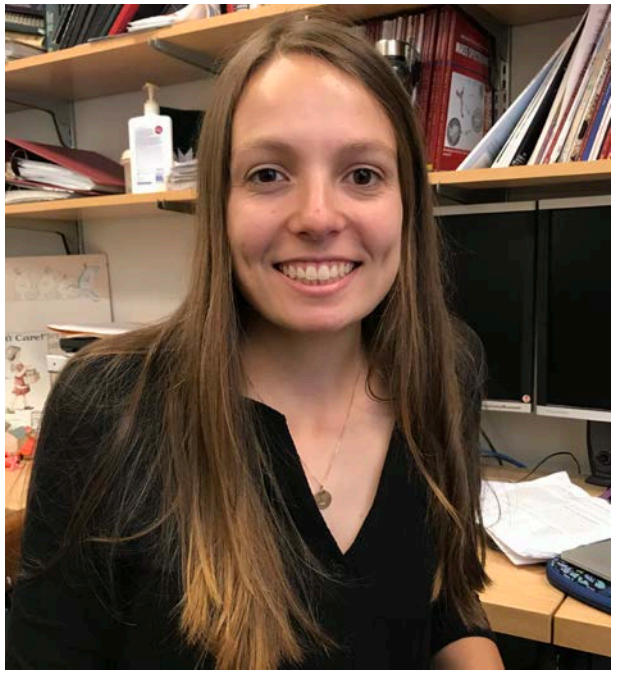
The ultimate burden of REACH depends on how petrochemicals are handled

Food for Thought ...

ALTEX 2018, 35:275-305

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

Lucy Meigs [1,2], Lena Smirnova [2], Costanza Rovida [3], Marcel Leist [3] and Thomas Hartung [2,3]



Tox: $18.6 billion (14,4 *in vitro*, 4,2 *in vivo*)

40% of chemical industry is now in China
EU down from 35% to 15% in one decade

Sales in Europe 25.9% petrochemicals

REACH: registered as categories with unclear acceptability

REACH: Data-rich substances registered 2010 and 2013:

75% of dossiers use read-across

Other alternatives hardly used

Expertise in industry low

Low acceptance by EChA

Read-across

Data gap filling concluding from (structurally) similar chemicals

Category approach

Test only representatives of a group of similar chemicals or complex mixtures

Data gap filling from similar chemicals

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 Patlewicz [1], Nicholas Ball [2], Richard A. Becker [3], Ewan D. Booth [4], Mark T. D. Cronin [5], Dinant Kroese [6], David Steup [7], Ben van Ravenzwaay [8] and Thomas Hartung [9]

transatlantic think tank for toxicology

t4 report*

Toward Good Read-Across Practice (GRAP) Guidance

Nicholas Ball [15], Mark T. D. Cronin [2*], Jie Shen [3*], Karen Blackburn [4], Ewan D. Booth [5], Mounir Bouhifd [6], Elizabeth Donley [7], Laura Egnash [7], Charles Hastings [8], Daland R. Juberg [1], Andre Kleensang [9], Nicole Kleinstreuer [9], E. Dinant Kroese [10], Adam C. Lee [11], Thomas Luechtefeld [6], Alexandra Maertens [6], Sue Marty [1], Jorge M. Naciff [4], Jessica Palmer [7], David Pamies [6], Mike Penman [12], Andrea-Nicole Richarz [2], Daniel P. Russo [13], Sharon B. Stuard [4], Grace Patlewicz [14], Bennard van Ravenzwaay [10], Shengde Wu [4], Hao Zhu [13] and Thomas Hartung [6,15]*

transatlantic think tank for toxicology

t4 report*

Supporting Read-Across Using Biological Data

Hao Zhu [1], Mounir Bouhifd [2], Elizabeth Donley [3], Laura Egnash [3], Nicole Kleinstreuer [4], E. Dinant Kroese [5], Zhichao Liu [6], Thomas Luechtefeld [2], Jessica Palmer [3], David Pamies [2], Jie Shen [7], Volker Strauss [8], Shengde Wu [9] and Thomas Hartung [2, 10]

ALTEX 2018, 35:413-419

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 Maertens[1]

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

ECHA

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

AAT

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.”

ECHA

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

AAT

Natural language processing
(Feb 2016)
&
Web app
ToxTRACK

UL

REACHAcross™

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

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

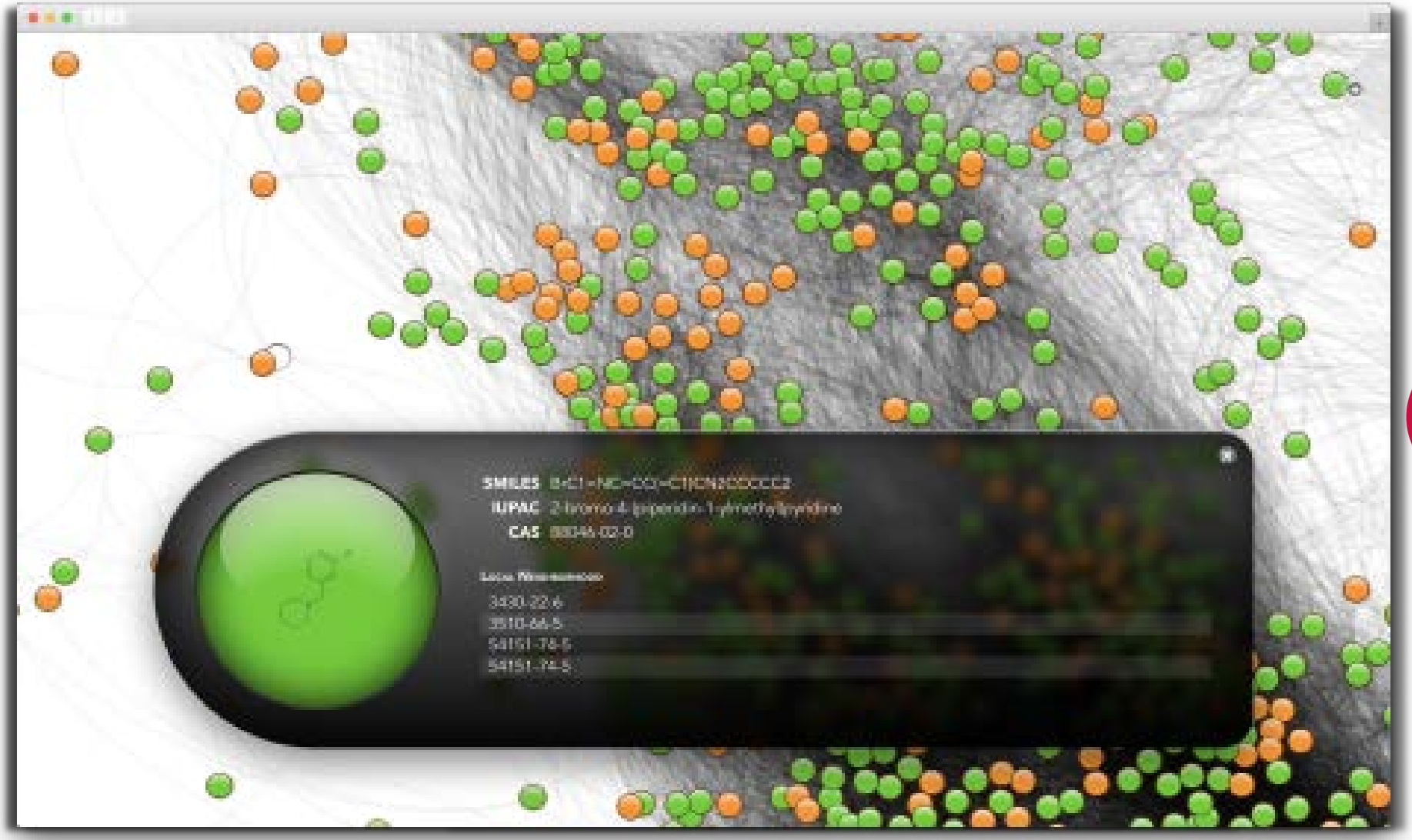
Similarity = proximity

ARTIFICIAL INTELLIGENCE
0,5 BILLION CALCULATIONS PER PREDICTION
+ CERTAINTY





CHEMICAL UNIVERSE – CURRENT DATABASE



REACHAcross™

UL COLLABORATION

10 million compounds
50 trillion comparisons

2 days on Amazon cloud server

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

Cite this: DOI: 10.1039/c8tx00051d

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

Thomas Luechtefeld,[a] Craig Rowlands[b] and Thomas Hartung *[a]

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

The next level: DATA FUSION

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

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

Toxicological Sciences, kfy152, https://doi.org/10.1093/tox

Published: 11 July 2018

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International journal of science

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NEWS · 11 JULY 2018

Software beats animal tests at predicting toxicity of chemicals

Machine learning on mountain of safety data improves automated assessments.

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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

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 Sensitisation Binary	7,670	80	96	88	84

Coverage 100% !

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

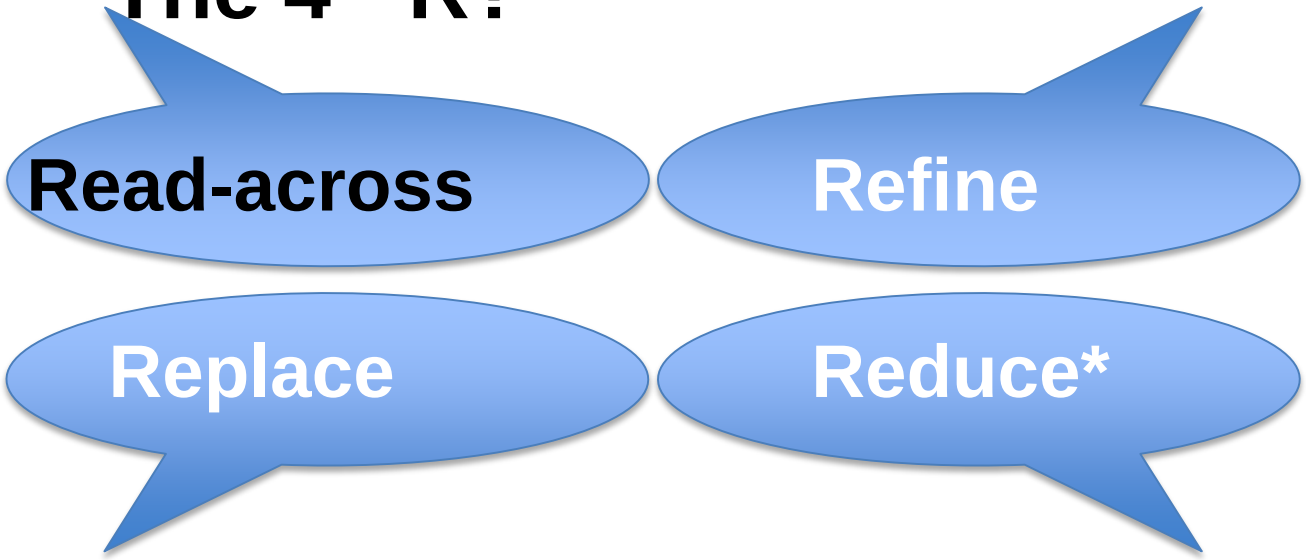
The 9 tests consume 5-600.000 animals in Europe per year

Formal validation will have to show,

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

The 4R

The 4th R?



***pesticides**

Does not (yet) help for complex (expensive) endpoints

Usefulness for mixtures only starting to be explored

Mixture Toxicology Collaboration

- **Failure of many alternatives for mixtures**
- **Petrochemicals and REACH**
- **Cosmetic end-product testing**
- **Pesticide minimal formulation changes**

We need new approaches

transatlantic think tank for toxicology

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

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

t4 Report*

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

David A. Basketter [1,§], Harvey Clewell [2,§], Ian Kimber [3,§], Annamaria Rossi [4,§], Bas Blaauboer [5], Robert Burrier [6], Mardas Daneshian [7], Chantra Eskes [8], Alan Goldberg [9], Nina Hasiwa [10], Sebastian Hoffmann [11], Joanna Jaworska [12], Thomas B. Knudsen [13], Robert Landsiedel [14], Marcel Leist [15], Paul Locke [9], Gavin Maxwell [16], James McKim [17], Emily A. McVey [18], Gladys Ouédraogo [19], Grace Patlewicz [20], Olavi Pelkonen [21], Erwin Roggen [22], Costanza Rovida [23], Irmela Ruhdel [24], Michael Schwarz [25], Andreas Schepky [26], Greet Schoeters [27], Nigel Skinner [28], Kerstin Trentz [29], Marian Turner [30], Philippe Vanparys [31], James Yager [32], Joanne Zurlo [9], and Thomas Hartung [33,§]

ALTEX 2012, 29:3-89

Brussels, March 2012

DC, May 30-31, 2013

Stakeholder Fora

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

Joint CAAT – BASF - EU-ToxRisk think tank

May 15th to 17th 2017

on **“Alternative Approaches for Developmental and Reproductive Toxicity (DART) Testing”**

in Konstanz, Germany

Universität Konstanz

JOHNS HOPKINS BLOOMBERG SCHOOL of PUBLIC HEALTH

Report in preparation

EUTOXRISK

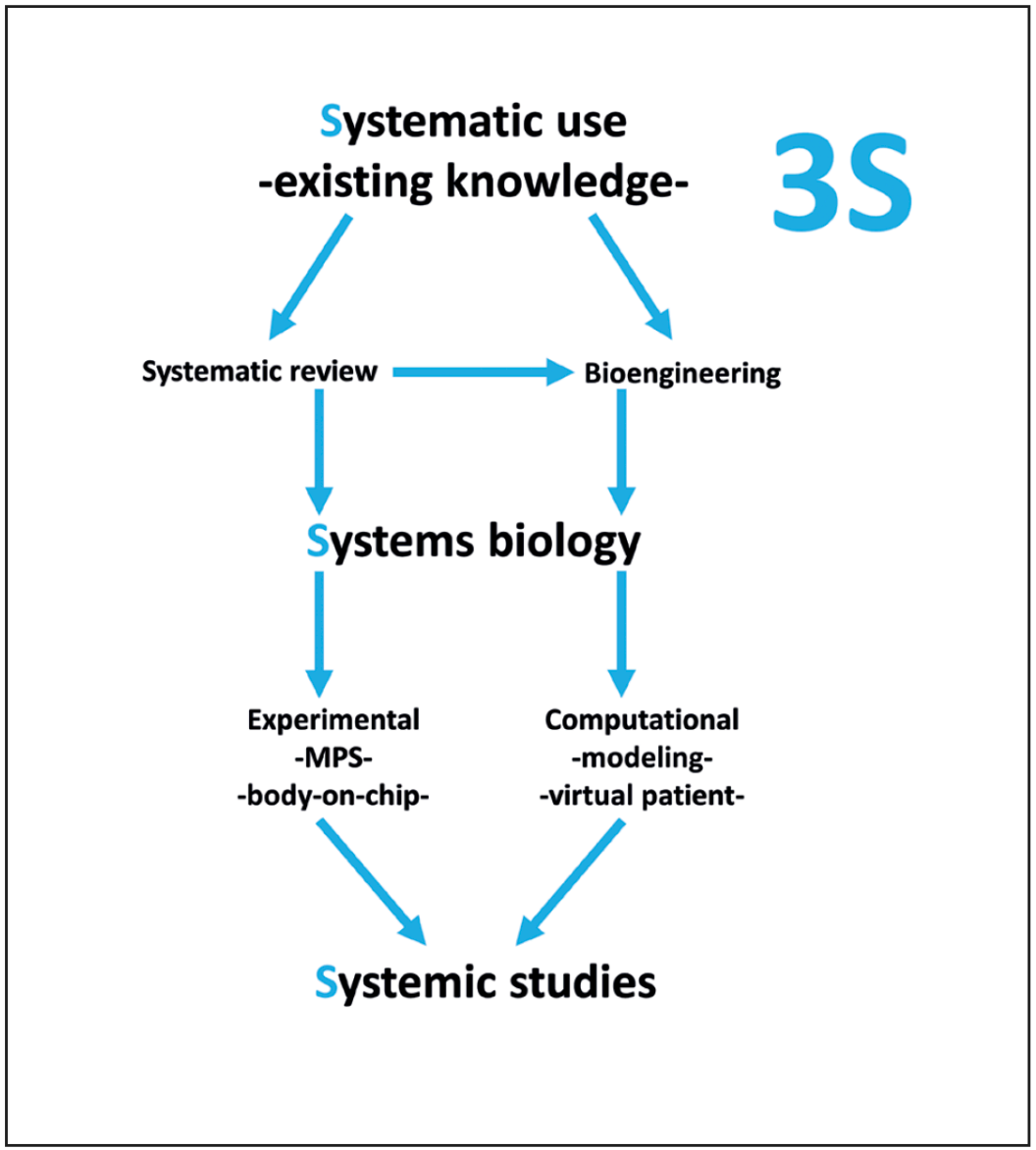


Fig. 1: The 3S approach to study systemic phenomena

ALTEX 2018, 35:139-162

"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 Smirnova [1], Nicole Kleinstreuer [2], Raffaella Corvi [3], Andre Levchenko [4], Suzanne C. Fitzpatrick [5] and Thomas Hartung [1,6]

Too expensive

Only for individual chemicals

NOEL

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

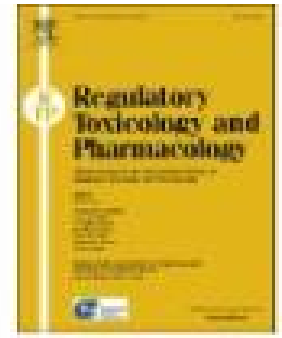
Thomas Hartung

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]

ALTEX 2017, 34:3-21

Food for Thought ...

The Need for Strategic Development of Safety Sciences

Francois Busquet [1] *and Thomas Hartung* [1,2]

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

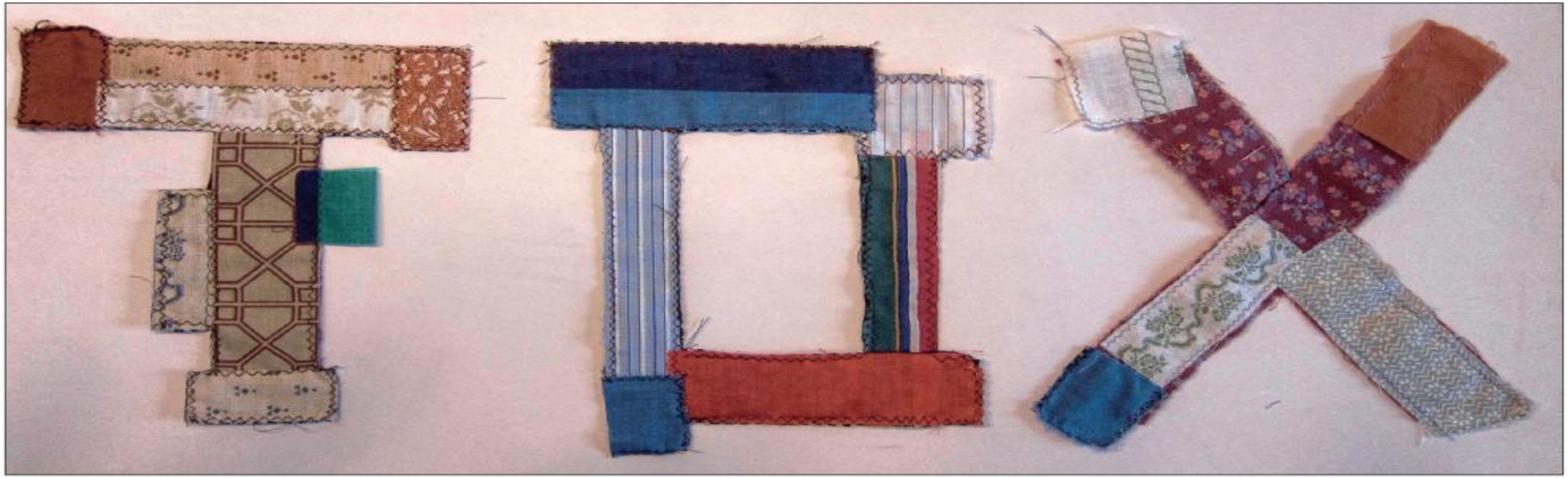


Fig. 1: The patchwork building of toxicology
(courtesy of Ingrid Hartung, Solingen, Germany)

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

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