Toxicology in the 21st Century

Thomas Hartung

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About 1/3 funding each from industry, philanthropy and research funding

ALLERGAN

RIFM

IFRA INTERNATIONAL FRAGRANCE ASSOCIATION

BDF Beiersdorf

Johnson & Johnson

Agilent Technologies

NOVARTIS

Abbott A Promise for Life

Pfizer Working together for a healthier world™

ExxonMobil

Boehringer Ingelheim

L'ORÉAL

Roche

P&G

Unilever

BASF The Chemical Company

The Bernice Barbour Foundation

DOERENKAMP-ZBINDEN Foundation

The Esther A. & Joseph Klingenstein Fund, Inc.

…and individuals

EUROPEAN COMMISSION

FDA

NATIONAL INSTITUTES OF HEALTH

DEFENSE THREAT REDUCTION AGENCY UNITED STATES OF AMERICA

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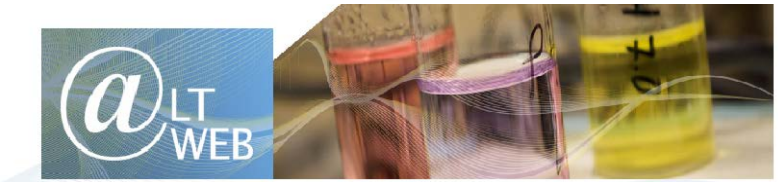
CAAT Programs



- Information and communication
- Policy and Outreach Program
- Refinement Program
- Transatlantic Think Tank of Toxicology (t4)
- Evidence-based Toxicology
- Grants Programs
- Education
- Research

Examples of food related activities

Workshop
Tox-21 beyond chemicals
June, 2012

Food Information Day

Current Issues and Future Prospects of Food Safety, Risk Assessment, Risk Management, International Regulations, and Animal Health

Linkages & Separation of Risk Assessment & Risk Management
Herman Köeter, Orange House Partnership

Animal Health & Welfare – risk assessment & impact on legislation & regulatory practices – a US-EU comparison
Jordi Serratosa, European Food Safety Commission

Pesticide Law – EU and New Developments

Pesticides – Safety Assessment / Risk Assessment
- Ann Blacker, Bayer Crop Sciences
- Douglas Wolf, US Environmental Protection Agency

Opportunities for the 3Rs in Food Risk Assessment
- Timothy Pastoor, Syngenta, Ltd.
- Charles Hastings, BASF
- Thomas Hartung, Johns Hopkins Center for Alternatives to Animal Testing

Tuesday, November 15, 2011
9:00 am – 3:30 pm (includes lunch)
E2030 - Feinstone Hall

Co-sponsored by EHS Center for Alternatives to Animal Testing and the Orange House Partnership

Registration Information: http://caat.jhsph.edu/programs/workshops/foodinformation.html
Contact: Marilyn Principe mprincip@jhsph.edu or phone: 410 614 4919

For disability access information or listening devices, please contact the Office of Support Services at 410-955-1197, or on the Web at www.jhsph.edu/SupportServices.
EO/AA

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100,000+ chemicals in consumer products

Infectious pathogens,
e.g. Salmonella, Campylobacter,
Hepatitis

Food additives,
ca. 3,000 substances

Toxins, e.g. botulinum,
marine biotoxins,
mycotoxins, aflatoxin

Plant protection products,
ca. 1,000 substances

Contaminants,
e.g. dioxins, heavy metals,
acrylamide

Lack of essential
nutrients

Unhealthy nutrients
and calorie overload

Food allergens

Source of picture: animated fast-food superheroes of "Aqua Teen Hunger Force" from Cartoon Network

Fig. 1: Food safety concerns

Hartung and Koeter, ALTEX 2008, 259-264.

Consumer are little aware of animal testing for food

Strong discrepancies for e.g. food additives vs. pesticides

Evident data gaps, But traditional approaches not suitable

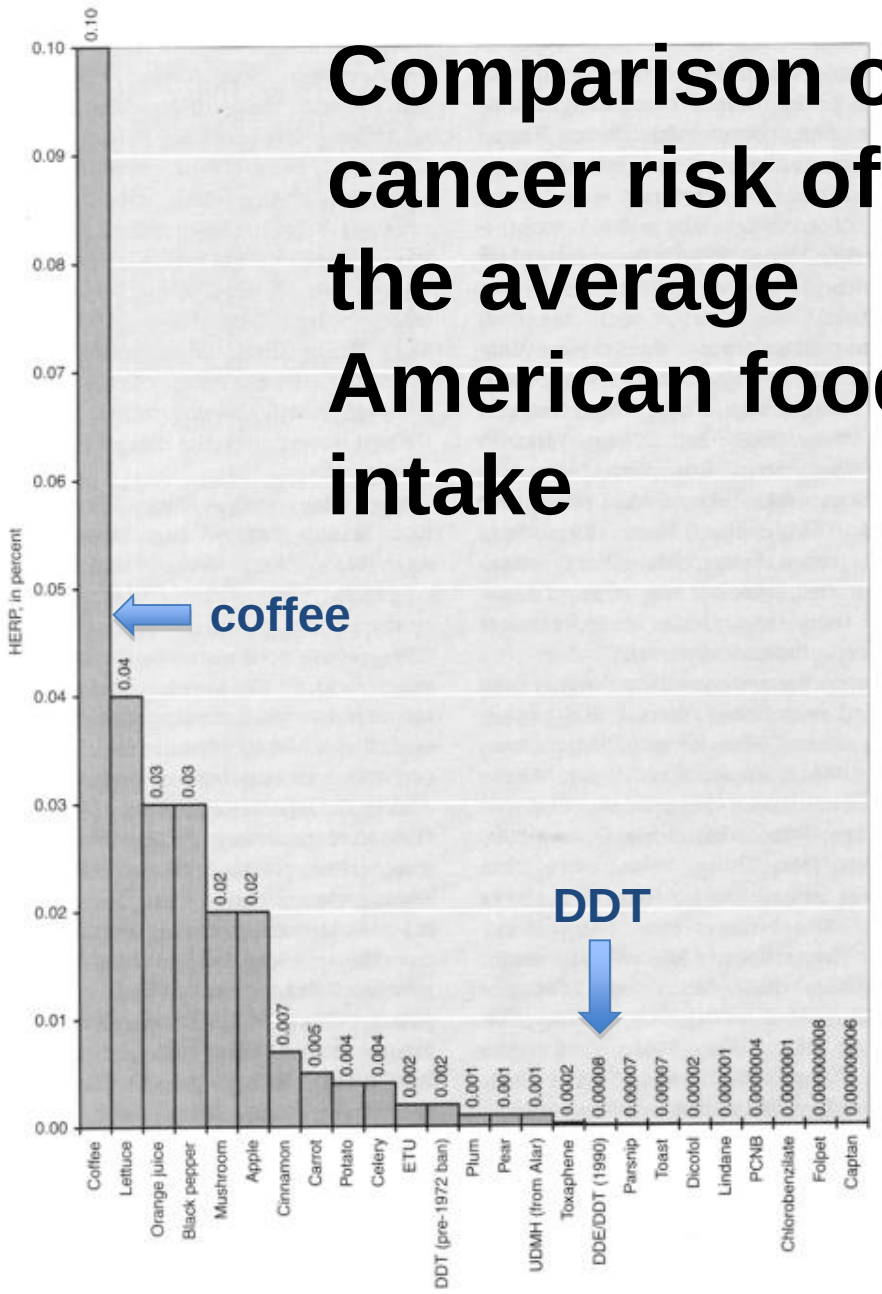
Source:
B. Ames & L. Gold

Alcohol 36-times more dangerous than coffee intake

When using the same threshold levels as for TCDD (dioxin):

One beer in 345 years

Comparison of cancer risk of the average American food intake



ure 127 Comparison of relative cancer risk (HERP) of the average American daily intake of various foods and hetic pesticides. The alcohol intake of the average adult American is equivalent to 1.7 beers or a HERP of 3.6

R22 harmful if swallowed

(LD_{50} = 150-200mg/kg in rats)

R 36 irritant to eyes

R 37 respiratory irritant

R 38 irritant to skin

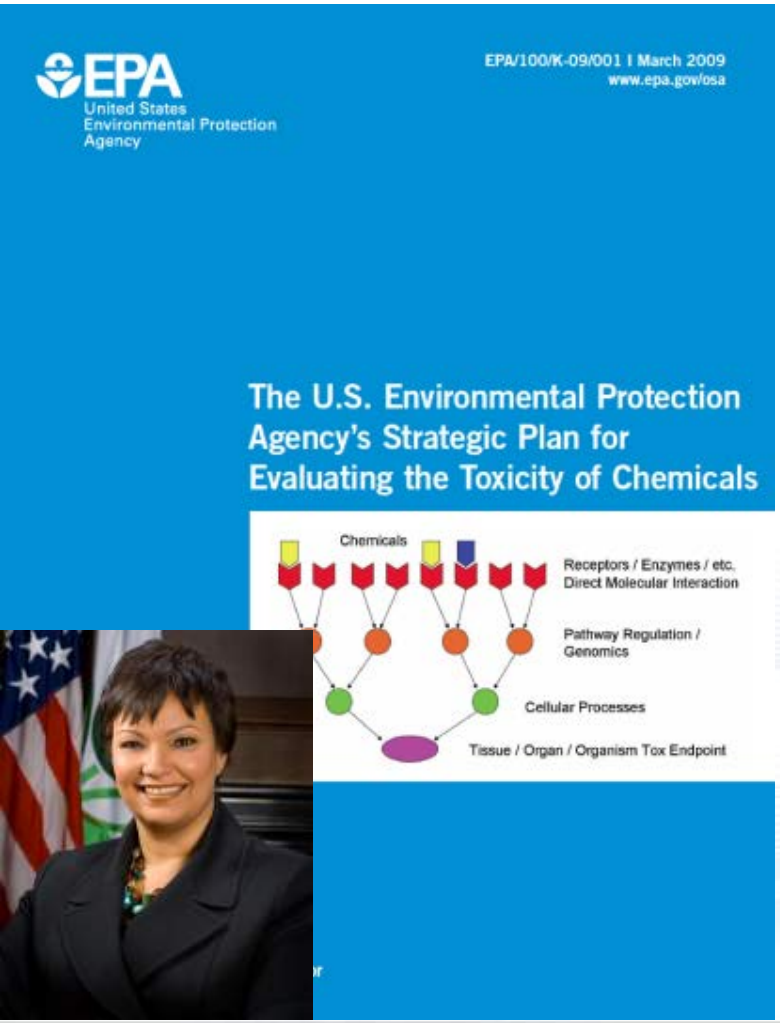
Not carcinogenic,
but co-carcinogen (promotor)

Unclear mutagenicity

Embryonic malformations in cat, dog, rat, mice, rabbit, monkey

Unlikely to be brought to the market today

NAS vision report Tox-21c



"With an advanced field of regulatory science, new tools, including functional genomics, proteomics, metabolomics, high-throughput screening, and systems biology, we can replace current toxicology assays with tests that incorporate the mechanistic underpinnings of disease and of underlying toxic side effects." **M.A. Hamburg, FDA 2011**

"We propose a shift from primarily in vivo animal studies to in vitro assays, in vivo assays with lower organisms, and computational modeling for toxicity assessments"
F. Collins, NIH, 2008

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Initiatives implementing Tox-21c

Organization	Approach	Purpose	Outcome
US EPA & Tox21 (ToxCast Program)	High-throughput testing	Chemical prioritization (initially)	"Biological signatures"
Hamner Institute	Case studies	"Just do it"	Proof-of-principle
NIH project (CAAT-US)	Pathway mapping	Pathway ID & annotation	Human Toxome

Tox-21c =

the end of GRAS

The new regulator

Tox-20c

EBT

Omics, high-content, HTS Bio-informatics & -engineering

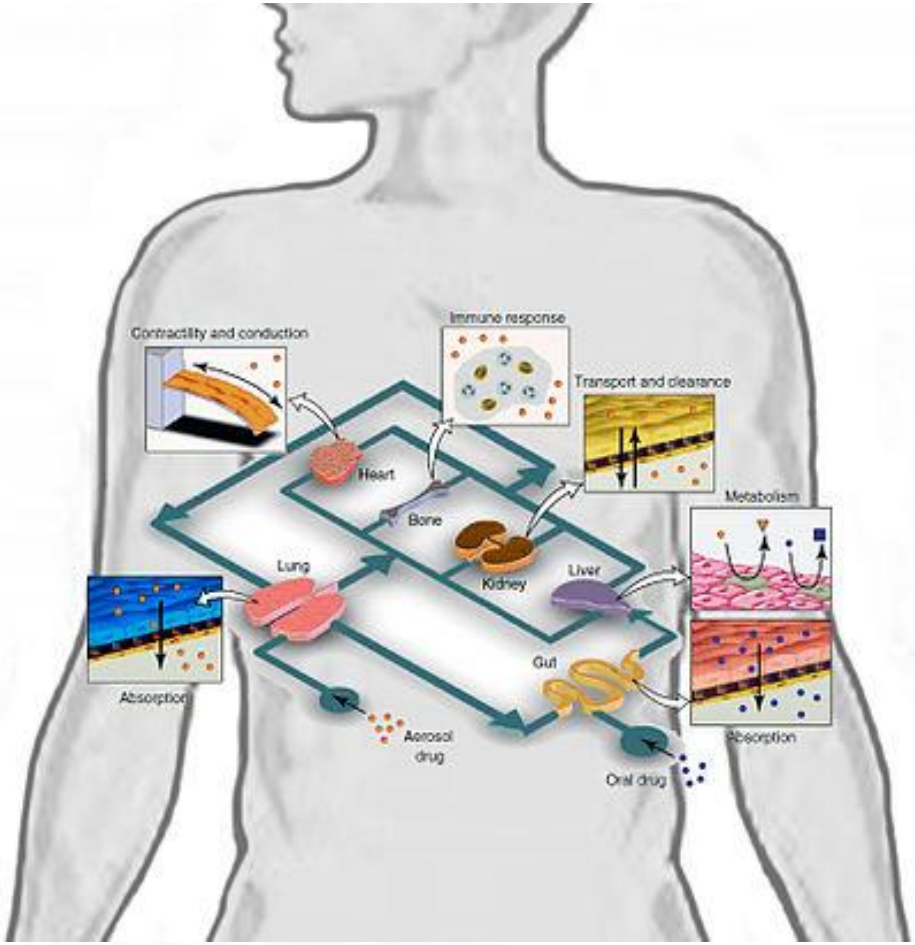
Tox-21c

Pathways of Tox (PoT)
Human Toxome

Integrated Testing Strategies
ITS

Organo-typic cultures
Human-on-Chip

Human on Chip Approach

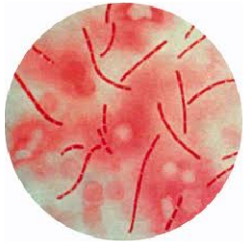


Could overcome many of these shortcomings, especially using stem cells

C. Zhang et al. (2009), "Towards a human-on-chip: Culturing multiple cell types on a chip with compartmentalized microenvironments"

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

CAAT
The Johns Hopkins Center for Alternatives to Animal Testing



CAAT Information Day
Tuesday, May 22, 2012
10:00 am – 4:30 pm
Sheldon Hall (W1214)
Johns Hopkins Bloomberg
School of Public Health
615 North Wolfe Street
Baltimore, MD

New Approaches to Assessing Countermeasures to Bioterrorism Agents

Speakers include:

George Korch (JHBSPH and US DHHS)
William C. (Clint) Florence (DTRA)
Donald Drake (Sanofi-Pasteur)
Marti Jett (US Army)
Anthony Bahinski (Wyss Institute, Harvard)
Sonia Grego (RTI International)
Lisa Hensley (US FDA)
Thomas Hartung (CAAT)

Registration fee (including lunch): $100 (free for the JHU community)
For registration and information, contact Marilyn Principe at mprincip@jhsph.edu

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Opportunities from countermeasures to bioterrorism

- **Funding program ($200 million) from NIH/FDA/DARPA/DTRA**
- **Need for predictivity, QA, validation**
- **Joint workshop 10 May 2013 FDA / NIH / DARPA / CAAT**

NCATS Catalyzing Innovation

DEFENSE THREAT REDUCTION AGENCY · UNITED STATES OF AMERICA

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InfoDay 22 May 2012

3D vs. 2D cultures

- **Increased cell survival**
- **Increased differentiation**
- **Increased cell – cell interaction**
- **Reproducing better the complexity of the organ**
- **Endpoints need optimization**
- **More complex – lower reproducibility**

Toxicology - $3 billion of testing to regulate $10 trillion of trade

Human-on-chip

Problems

- **Throughput** −
- **Costs** −
- **Predictivity** + ?
- **Too precautionary** ?
- **Animal use** +
- **New products** +
- **New hazards** +
- **Mixtures** −
- **Individuals** +

Tox-20c

EBT

Omics, high-content, HTS Bio-informatics & -engineering

Tox-21c

Pathways of Tox (PoT)
Human Toxome

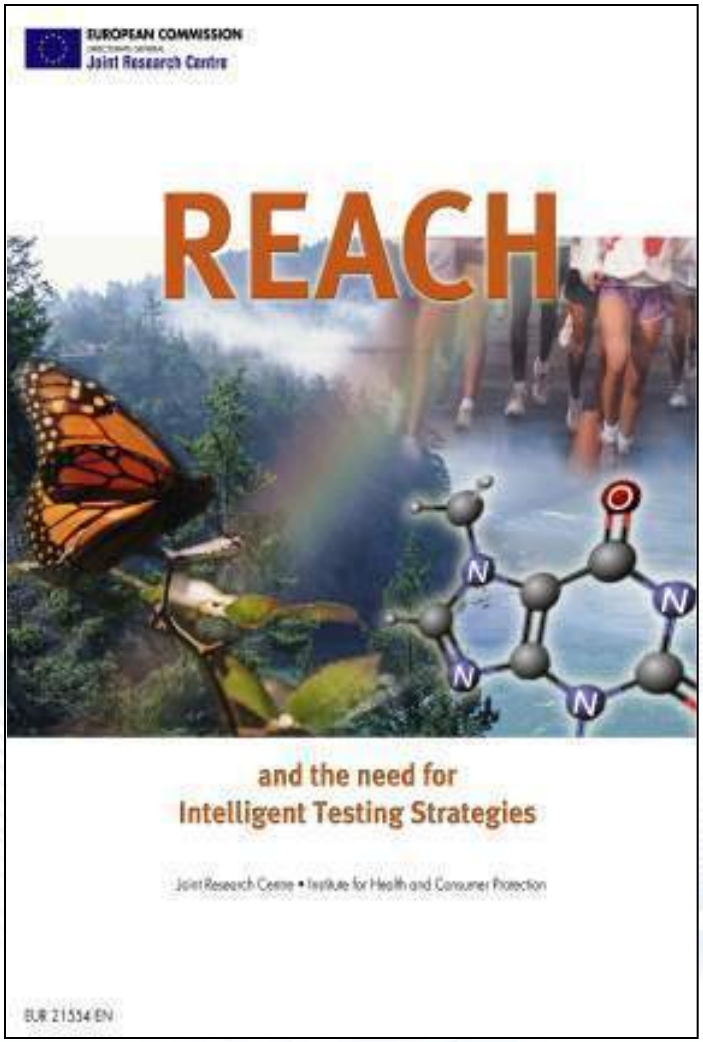
Integrated Testing Strategies
ITS

Organo-typic cultures
Human-on-Chip

Scientific roadmap for the future of animal-free systemic toxicity testing

US Stakeholder Forum 30-31 May 2013
Hosted by FDA CFSAN
Looking for further interested partners!!!

Integrated Testing Strategies



- **Key contribution to REACH implementation process**
- **Use of different informations, not stand-alone replacement**

Just became available (AltWeb or ALTEX website)

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

Thomas Hartung [1,2], *Tom Luechtefeld* [1], *Alexandra Maertens* [1], *and Andre Kleensang* [1]

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

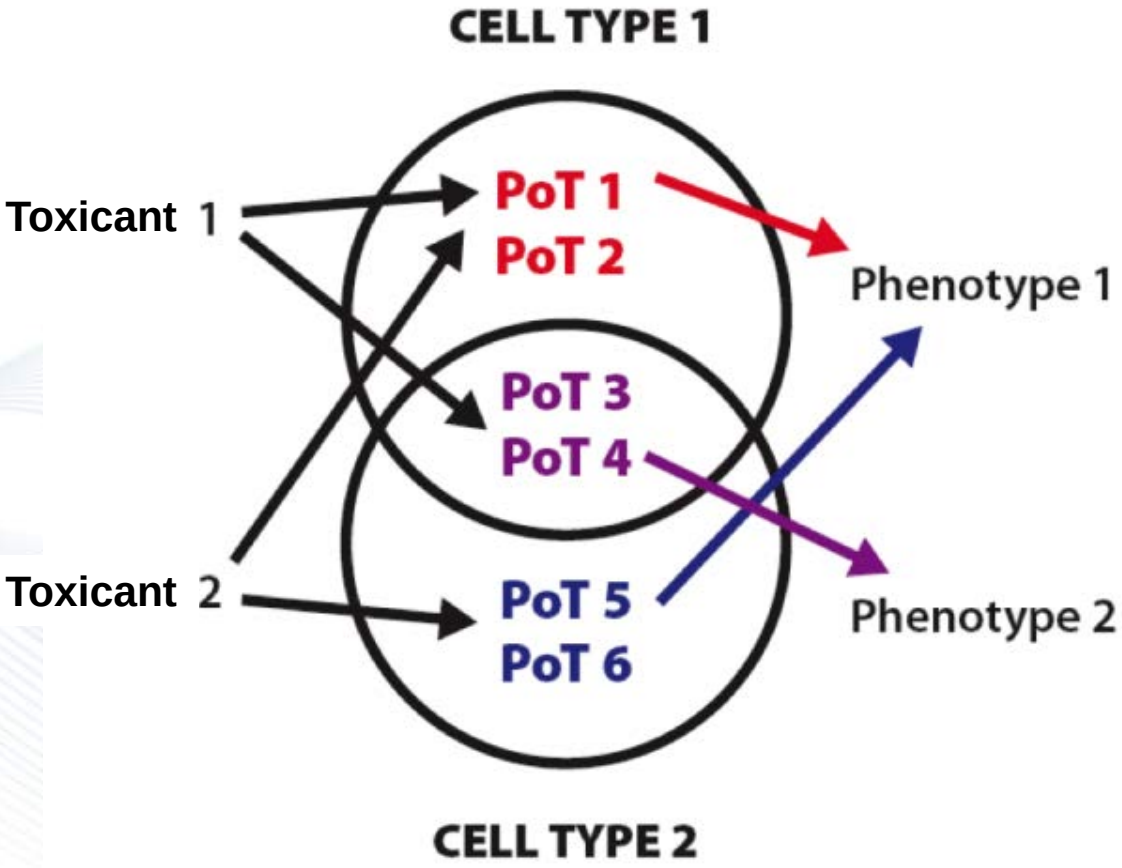
WoE, EBT, ITS…. Similar problems, but not the same

all: quality and data integration problem

EBT/WoE retrospective -- ITS prospective

WoE pragmatic -- EBT / ITS formalized

The concept of (finite number of) pathways of toxicity



Annotation to:

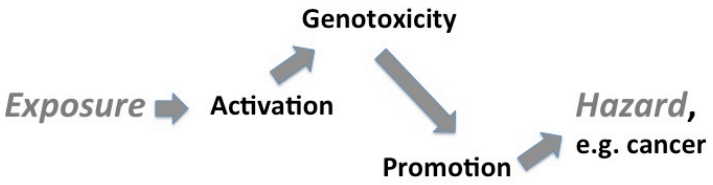
- **Hazard**
- **Toxin (class)**
- **Cell type**
- **Species**

Comprehensive list (Human Toxome) → **Negatives**

A) Phenomenological Toxicology

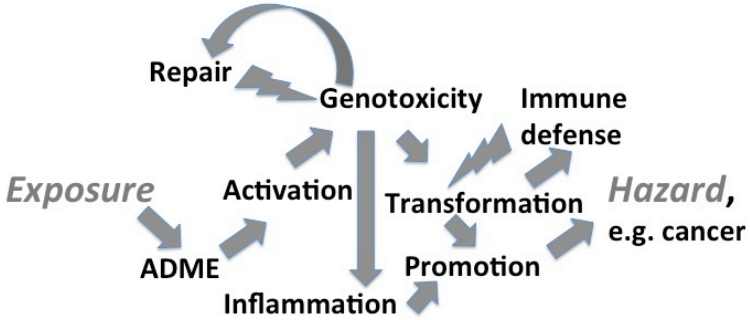
Causality supported by correlation and e.g. Bradford-Hill criteria

B) Mode of Action Toxicology (e.g. Adverse Outcome Pathways)



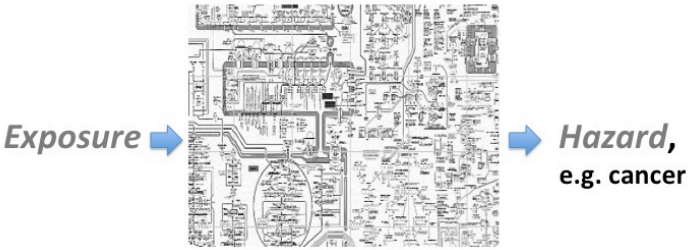
Causality supported by experimental intervention, e.g. Koch-Dale approaches

C) Mechanistic Toxicology (toxicity pathways)

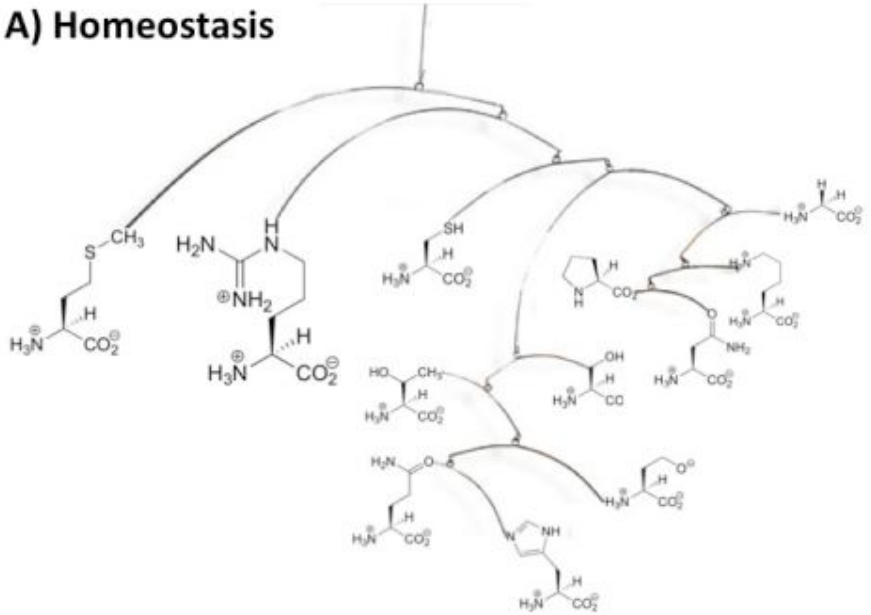


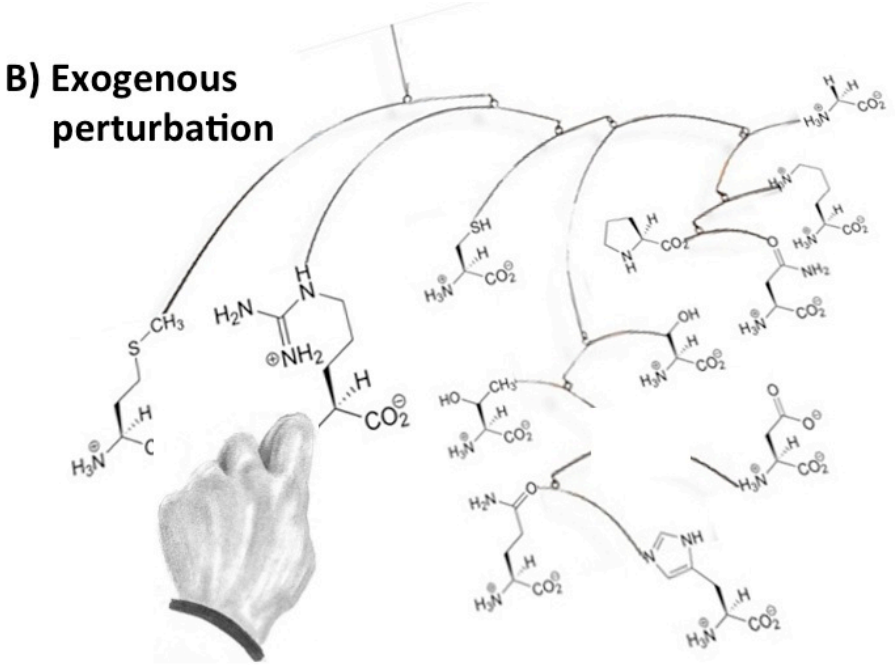
Causality supported by simulation and other bioinformatic tools

D) Systems Toxicology (resolution on PoT and fluxes)



Systems Toxicology approaches





Use for PoT identification:

- Homeostasis under stress, i.e. signatures of tox
- Critical cell infrastructures
- Network knowledge
- Reference models
- Reference toxicants

NIH Transformative Research Grant: *Mapping the Human Toxome by Systems Toxicology*

Consortium:

- **Johns Hopkins (Hartung / Yager)**
- **Brown (Boekelheide)**
- **The Hamner (Andersen)**
- **Georgetown (Fornace)**
- **Agilent (Rosenberg)**
- **EPA ToxCast (Kavlock, Dix)**

Mapping PoT from metabolomics and transcriptomics

In vitro systems

- Toxicological endpoints
- Model Systems Characterization
- In vitro treatments

omics data generation

- HC omics data Metabolomics &Transcriptomics

Software tools

- Software for Statistics and Pathways
- Visualization Tools

Pathways of Toxicity

- Signature of Toxicity Analysis
- PoT Concept Development
- Pathway Validation
- IV-IV Extrapolation
- Toxcast, other data

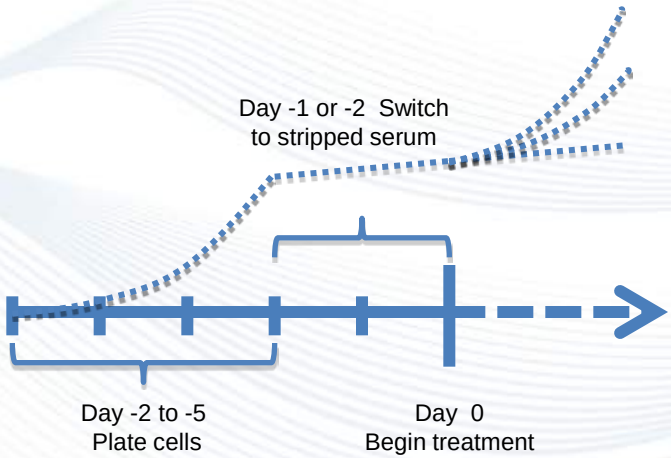
Human Toxome Database

(Pre-)Validated work

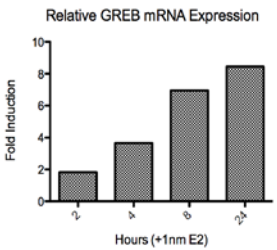
- **Robust protocols, good cell models**
- **Regulatory acceptance available or in progress**
- **Available reference substances**
- **Thresholds of adversity defined**

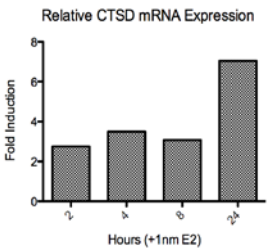
In vitro systems

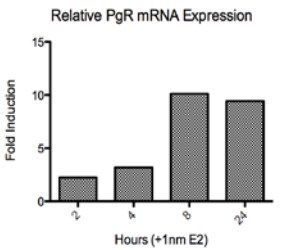
- **MCF-7 cells (currently undergoing validation by ICCVAM) &**
- **Initial set of endocrine disrupting chemicals selected from a priority list of 53 reference compounds identified by ICCVAM**



Cell culture SOP

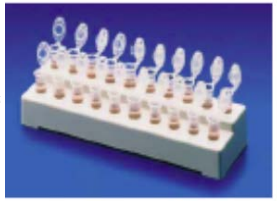






Gene Expression – Timecourse
1nM estradiol – 2, 4, 8, and 24 hours

MCF-7 cell culture and E2 exposure



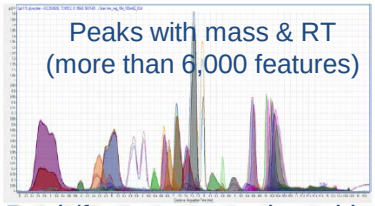
Sample prep and metabolite extraction

Data acquisition (Q-Tof)

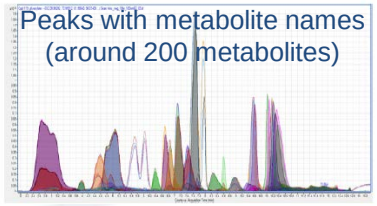
omics data generation

Untargeted

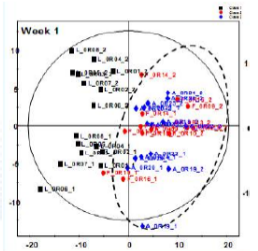
Targeted



Peak/feature extraction with NaÏve data mining algorithm

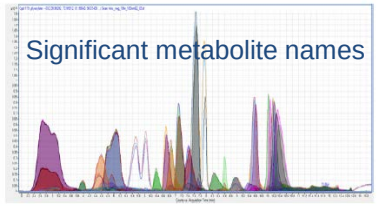


Metabolite peak extraction with database searching

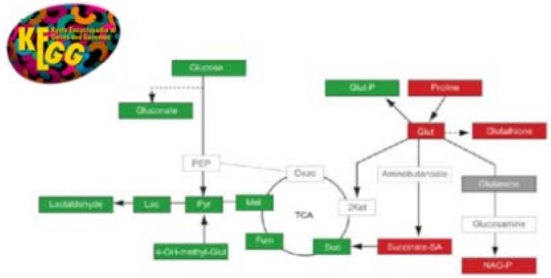


Bio-statistical analysis (e.g. ANOVA, PCA and clustering analysis)

Workflow for Metabolomics



Metabolites Identification (only for untargeted) and confirmation (for both approaches)



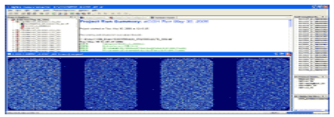
Data interpretation (e.g. Pathway analysis)

Software tools

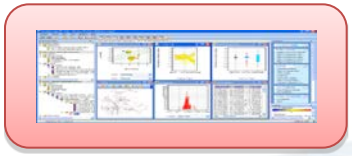
LC/MS
GC/MS

MassHunter Qual/Quant
ChemStation AMDIS

Microarrays



Feature Extraction



GeneSpring Platform

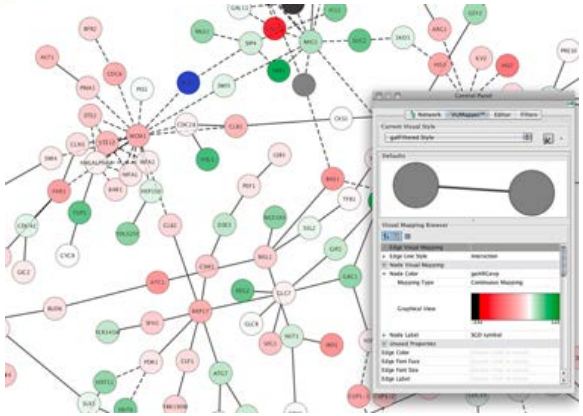
Biological
Pathways

NGS

Alignment to Reference Genome

Agilent Integrated Biology Workflows

Software tools

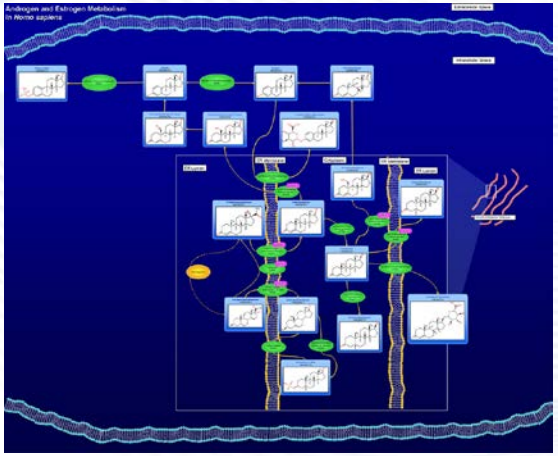


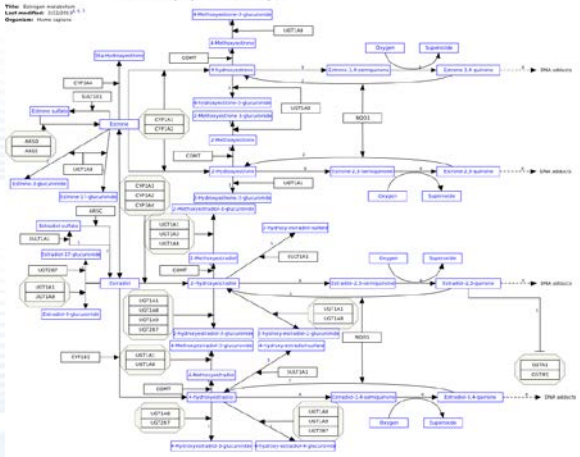
Cytoscape

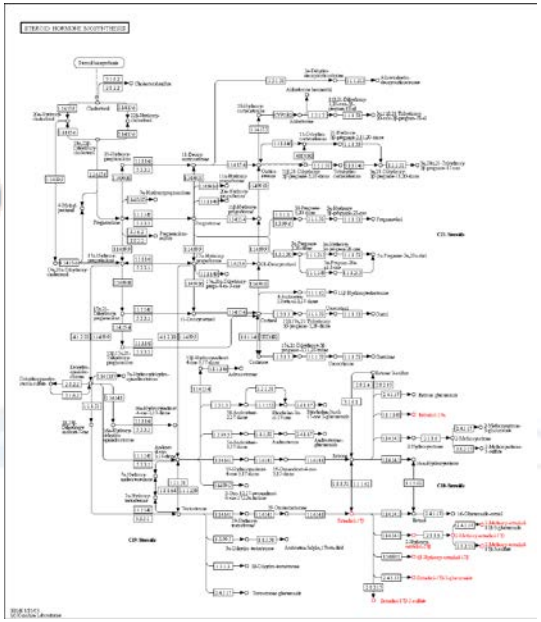
SMPDB
Small Molecule Pathway Database

WIKIPATHWAYS
Pathways for the People

KEGG
Kyoto Encyclopedia of Genes and Genomes

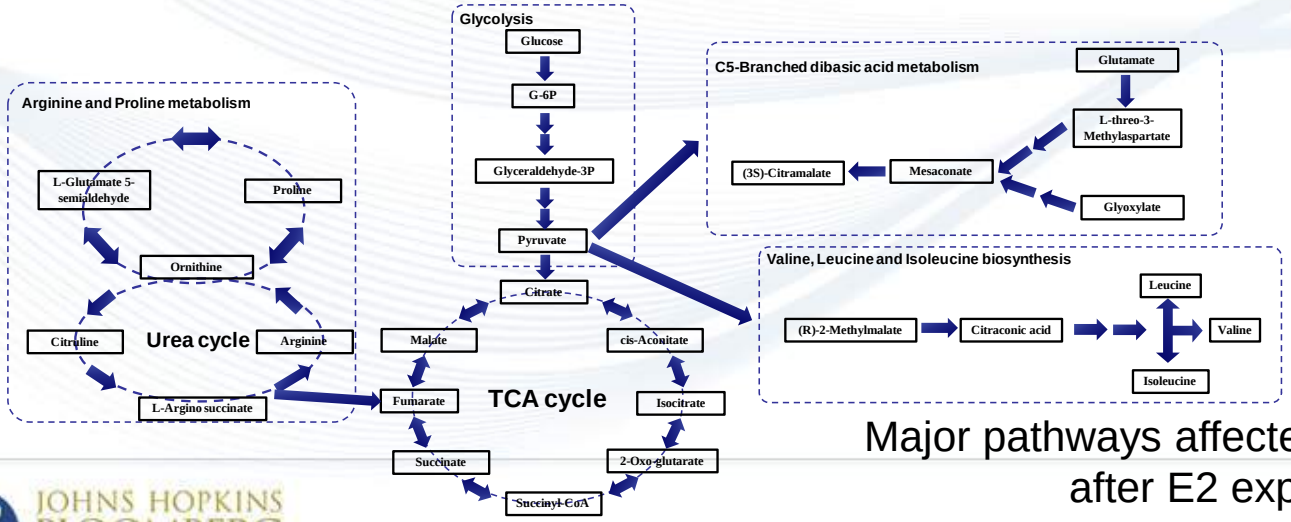






Significant Metabolites	Related Pathways
Malate	Citrate cycle (TCA cycle)
Fumarate	Citrate cycle (TCA cycle); Amino acid metabolisms
Aconitate	Citrate cycle (TCA cycle); C5-Branched dibasic acid metabolism
Pyruvate	Citrate cycle (TCA cycle); Glycolysis / Gluconeogenesis; Amino acid metabolisms
D-glyceraldehdye-3-phosphate	Glycolysis / Gluconeogenesis
Lactate	Glycolysis / Gluconeogenesis
Citraconic acid	Valine, leucine and isoleucine biosynthesis; C5-Branched dibasic acid metabolism
L-threo-3-Methylaspartate	C5-Branched dibasic acid metabolism
Glyoxylate	C5-Branched dibasic acid metabolism
(R)-2-Methylmalate	Valine, leucine and isoleucine biosynthesis; C5-Branched dibasic acid metabolism
Arginine	Arginine & Proline Metabolism
Valine	Valine, leucine and isoleucine biosynthesis
L-Glutamate 5-semialdehyde	Arginine & Proline Metabolism
Ornithine	Arginine & Proline Metabolism
Leucine/Isoleucine	Valine, leucine and isoleucine biosynthesis

Pathways of Toxicity



Major pathways affected in MCF-7 cells after E2 exposure

PROPOSAL FOR A TEMPLATE, AND GUIDANCE ON DEVELOPING AND ASSESSING THE COMPLETENESS OF ADVERSE OUTCOME PATHWAYS

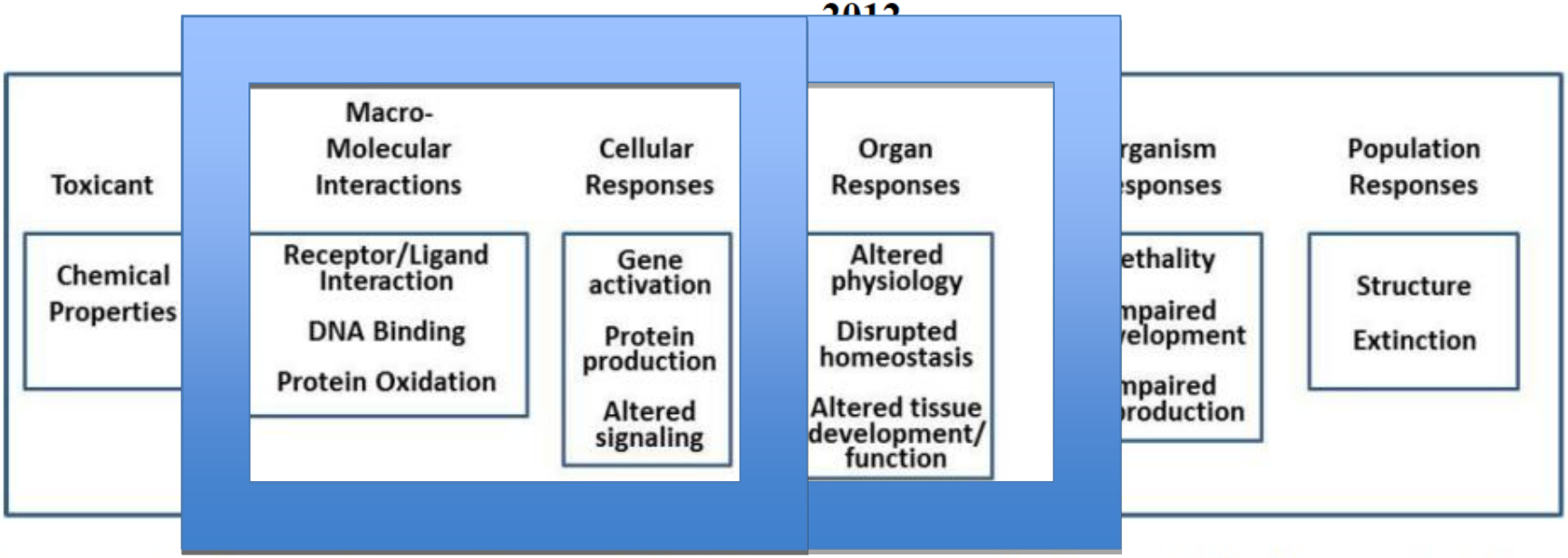
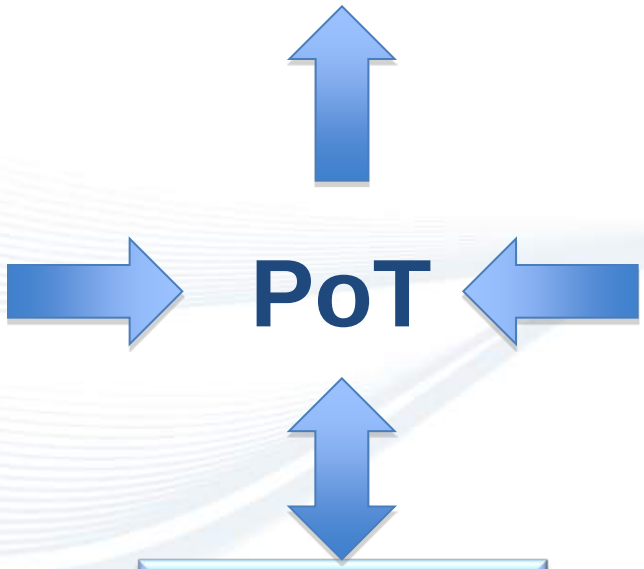


Figure1. A schematic representation of the Adverse Outcome Pathway (AOP) illustrated with reference to a number of pathways.

PoT

Workshop on the Concept and Tools for Pathways of Toxicity
October 10 -12, 2012, Baltimore, MD

Human Toxome database



Content side:
- Mol.biol.
- Biochem.
- Omics SoT
- Tox Mechan.

User side:
- Regulation
- Probabilistic RA
- Systems Toxicology
- Virtual patient

Existing databases

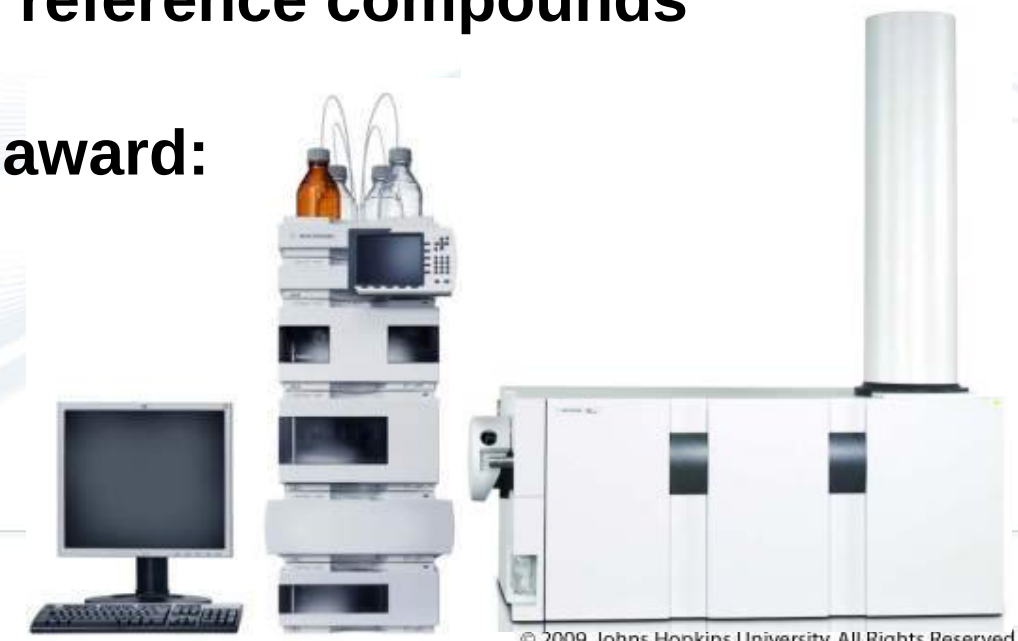
Metabolic profiling and Pathway of Toxicity identification in DNT

- **DNT (autism, hyperactivity);**
animal test $1.4 million, 1400 animals per substance

- **Identify metabolite changes relevant for neurodevelopment with reference compounds**

- **Agilent thought leader award: LC/MS system**

- **Integrate with genomics**



PoToMaC - The Pathways of Toxicty Mapping Center

European branch?

Transformative Research Grant:

Mapping the Human Toxome by Systems Toxicology

HUMAN TOXICOLOGY PROJECT CONSORTIUM

7 companies, 3 stakeholders

2 Mar 2012

"Driven both by legislative mandate and scientific need, a new suite of in vitro and cell culture-based animal-free methods are gaining a foothold in toxicology labs."

LIFE SCIENCE TECHNOLOGIES

Produced by the *Science*/AAAS Custom Publishing Office

Toxicology

Animal-Free Toxicology

Sometimes, in Vitro is Better

The next time you use shampoo, air freshener, or moisturizing cream, consider this: How do you know it's safe? In all likelihood, whatever toxicologic screening its component ingredients were subjected to involved laboratory animals, the method of choice for decades and the industry's reigning "gold standard." Yet as Bob Dylan once put it, the times, they are a-changing. Animal-based testing is expensive and time-consuming, morally and ethically troubling, and most significantly, often a poor predictor of human toxicity. Animals aren't going anywhere just yet. But their numbers are dropping. Driven both by legislative mandate and scientific need, a new suite of in vitro and cell culture-based animal-free methods are gaining a foothold in toxicology labs. **By Jeffrey M. Perkel**

One key player in the modernization of toxicology screening is automation.

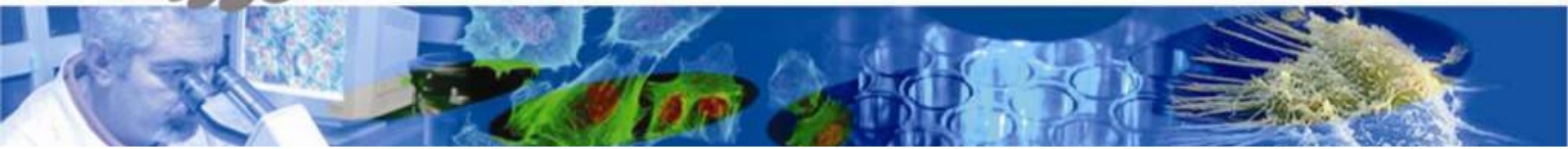
False
Evidence
Appearing
Real

The trap

Biological phenotyping (alerts) becomes available before we know how to interpret this in a risk context

EBT

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



Evidence-based Toxicology

"Evidence-based medicine goes toxicology!"

Hoffmann and Hartung "Toward an evidence-based toxicology", Human Exp. Tox., 2006

Mar 2011: US EBTC
Oct 2011: Secretariat at CAAT
Jan 2012: First conference hosted by EPA

Kick-off meeting of the Evidence-Based Toxicology Collaboration (EBTC) Europe

ebtc
Evidence-based Toxicology Collaboration

In conjunction with Eurotox Congress 2012 (Stockholm, Sweden)

June 17, 2012
15:30h - 17:30h

Radisson Blu Royal Viking Hotel • Vasagatan 1, Stockholm, Sweden
Complimentary Registration: http://www.ebtox.com

Johns Hopkins University Center for Alternatives to Animal Testing

EBT Collaboration Steering Committees

EBTC

www.ebtox.com

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Detection and sequencing of microRNA using MALDI time-of-flight mass spectr... | libres.uncg.edu/ir/uncg/f/Yang_uncg_0154M_10557.pdf | EBTC

RSS Twitter Facebook

ebtc
Evidence-based Toxicology Collaboration

About Us Contact Us Meetings & Symposia Steering Committee

What is Evidence-based Toxicology?

The Evidence-Based Toxicology (EBT) Collaboration has recently taken up the challenge of translating evidence-based approaches from medicine to toxicology. The Collaboration has closely coordinated steering committees in the US and Europe with members drawn from government agencies, academia, and industry. More. . . .

LATEST NEWS

US EBTC Receives Informal Tutorial on Systematic Reviews
The US EBTC Steering Committee held an informal tutorial on systematic reviews (SRs) on July 23, 2012 at Johns Hopkins S...

Kick-off meeting of the Evidence-Based Toxicology Collaboration (EBTC) Europe
In conjunction with Eurotox Congress 2012 (Stockholm, Sweden) June 17, 2012 | 15:30h - 17:30h Radisson Blu Royal Vi...

Definition of Validation

ALTEX 27 (2010) 253-263

Evidence-Based Toxicology – the Toolbox of Validation for the 21st Century?

Thomas Hartung
Johns Hopkins University, Bloomberg School of Public Health, Dept. Environmental Health Sciences, Center for Alternatives to Animal Testing (CAAT), Doerenkamp-Zbinden Chair for Evidence-based Toxicology, Baltimore, MD, USA, and Professor of Pharmacology and Toxicology, University of Konstanz, Germany

Knowledge:
- PoT
- MoA

Just became available (AltWeb or ALTEX website)

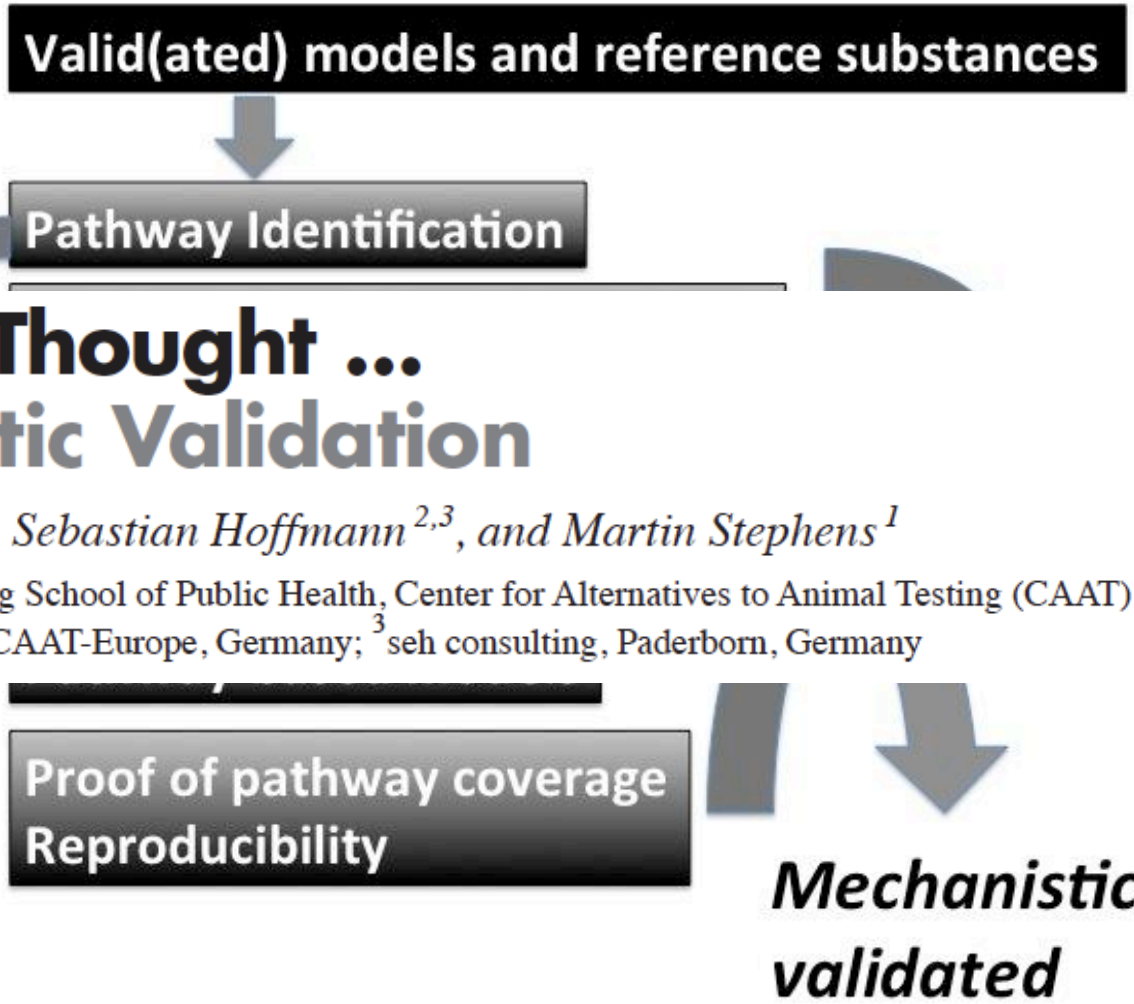
Workshop Report

Evidence-based Toxicology for the 21st Century: Opportunities and Challenges*

Martin L. Stephens [1], Melvin Andersen [2], Richard A. Becker [3], Kellyn Betts [4], Kim Boekelheide [5], Ed Carney [6], Robert Chapin [7], Dennis Devlin [8], Suzanne Fitzpatrick [9], John R. Fowle III [10], Patricia Harlow [11], Thomas Hartung [1], Sebastian Hoffmann [12], Michael Holsapple [13], Abigail Jacobs [11], Richard Judson [14], Olga Naidenko [15], Tim Pastoor [16], Grace Patlewicz [17], Andrew Rowan [18], Roberta Scherer [1], Rashid Shaikh [19], Ted Simon [20], Douglas Wolf [14], and Joanne Zurlo [1]

Perspectives on Validation of High-Throughput Assays Supporting 21st Century Toxicity Testing

Richard Judson [1], Robert Kavlock [1], Matthew Martin [1], David Reif [1], Keith Houck [1], Thomas Knudsen [1], Ann Richard [1], Raymond R. Tice [2], Maurice Whelan [3], Menghang Xia [4], Ruili Huang [4], Christopher Austin [4], George Daston [5], Thomas Hartung [6], John R. Fowle III [7], William Wooge [8], Weida Tong [9], and David Dix [1]



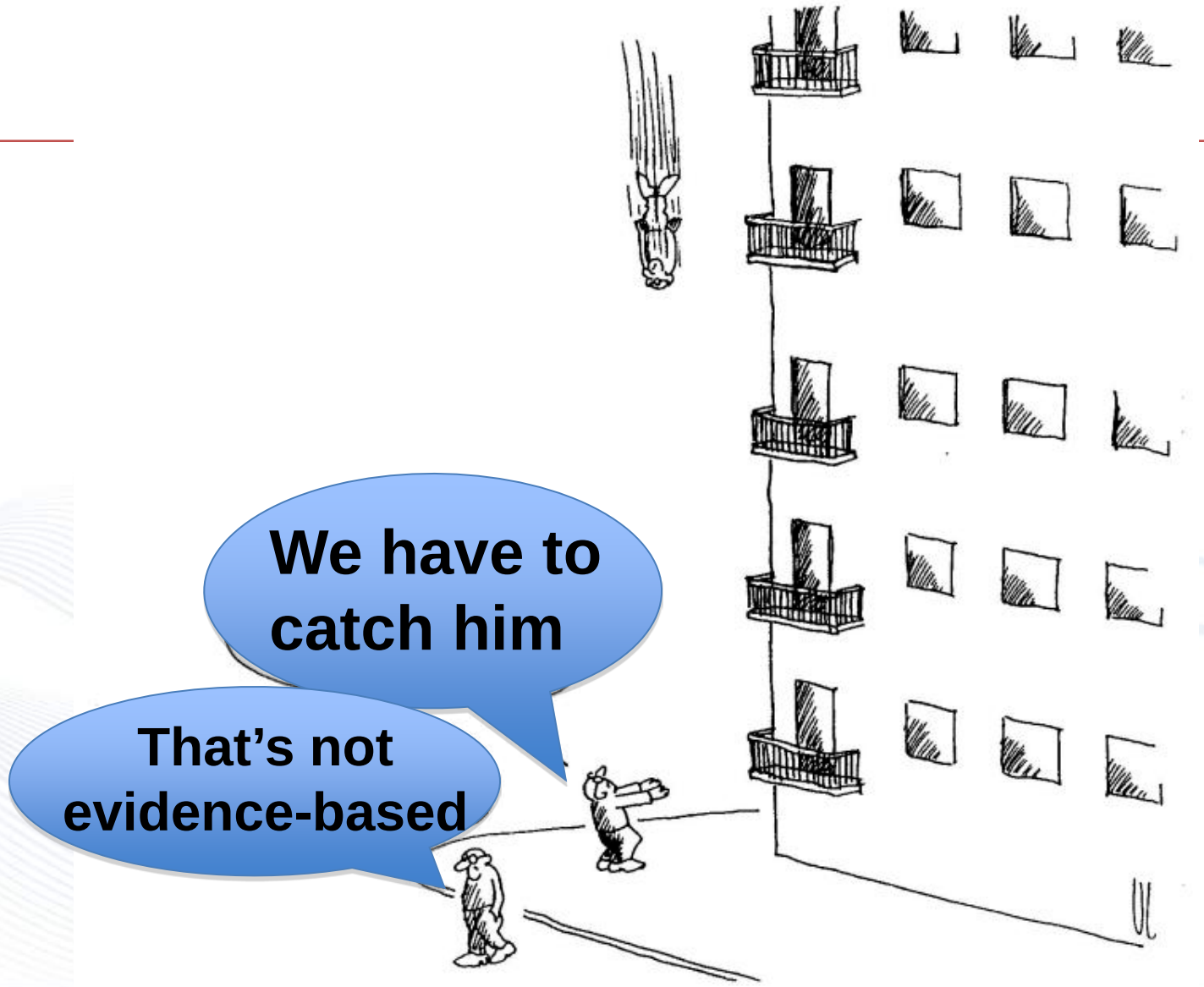
Food for Thought ...

Mechanistic Validation

Next ALTEX

Thomas Hartung [1,2], *Sebastian Hoffmann* [2,3], *and Martin Stephens* [1]

[1]Johns Hopkins Bloomberg School of Public Health, Center for Alternatives to Animal Testing (CAAT), Baltimore, MD, USA; [2]University of Konstanz, CAAT-Europe, Germany; [3]seh consulting, Paderborn, Germany



Johns Hopkins is the right environment for EBTC secretariat

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

John Maynard Keynes

(1883 - 1946)