Evidence-based methodologies in toxicology: Application to test methods comparison

U.S. Environmental Protection Agency Advancing Systematic Review for Chemical Risk Assessment Arlington, VA

December 17, 2015

Katya Tsaioun

EBTC Zebrafish Work Group

Evidence-based Toxicology Collaboration

Johns Hopkins School of Public Health

A CENTURY OF SAVING LIVES
MILLIONS AT A TIME

JOHNS HOPKINS
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ebtc
Evidence-based Toxicology Collaboration

About EBTC

What is EBTC?

The EBTC is a collaboration of science, regulatory and industry leaders

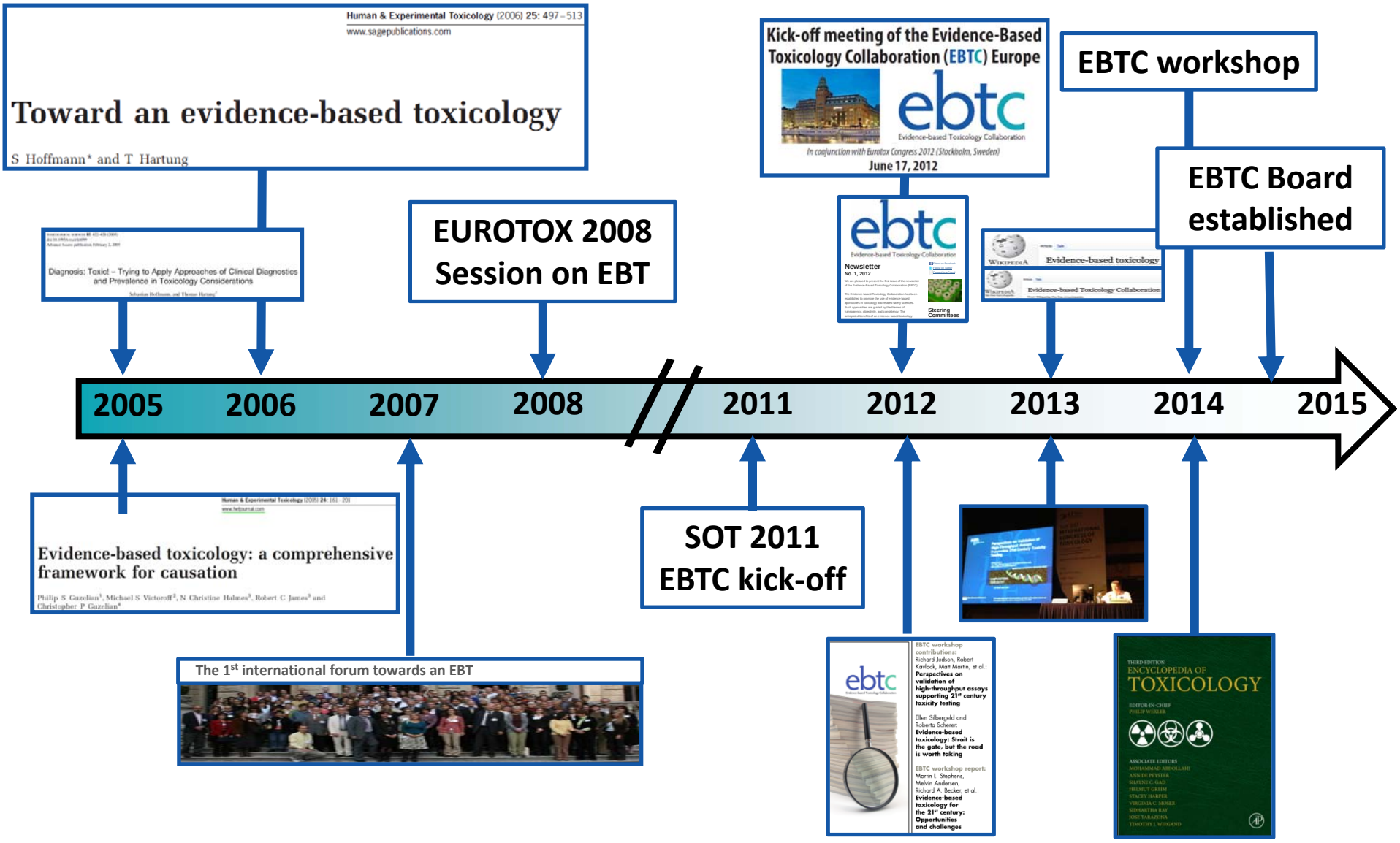
EBTC's Mission:

To bring evidence-based approaches to strengthen decision-making in safety sciences

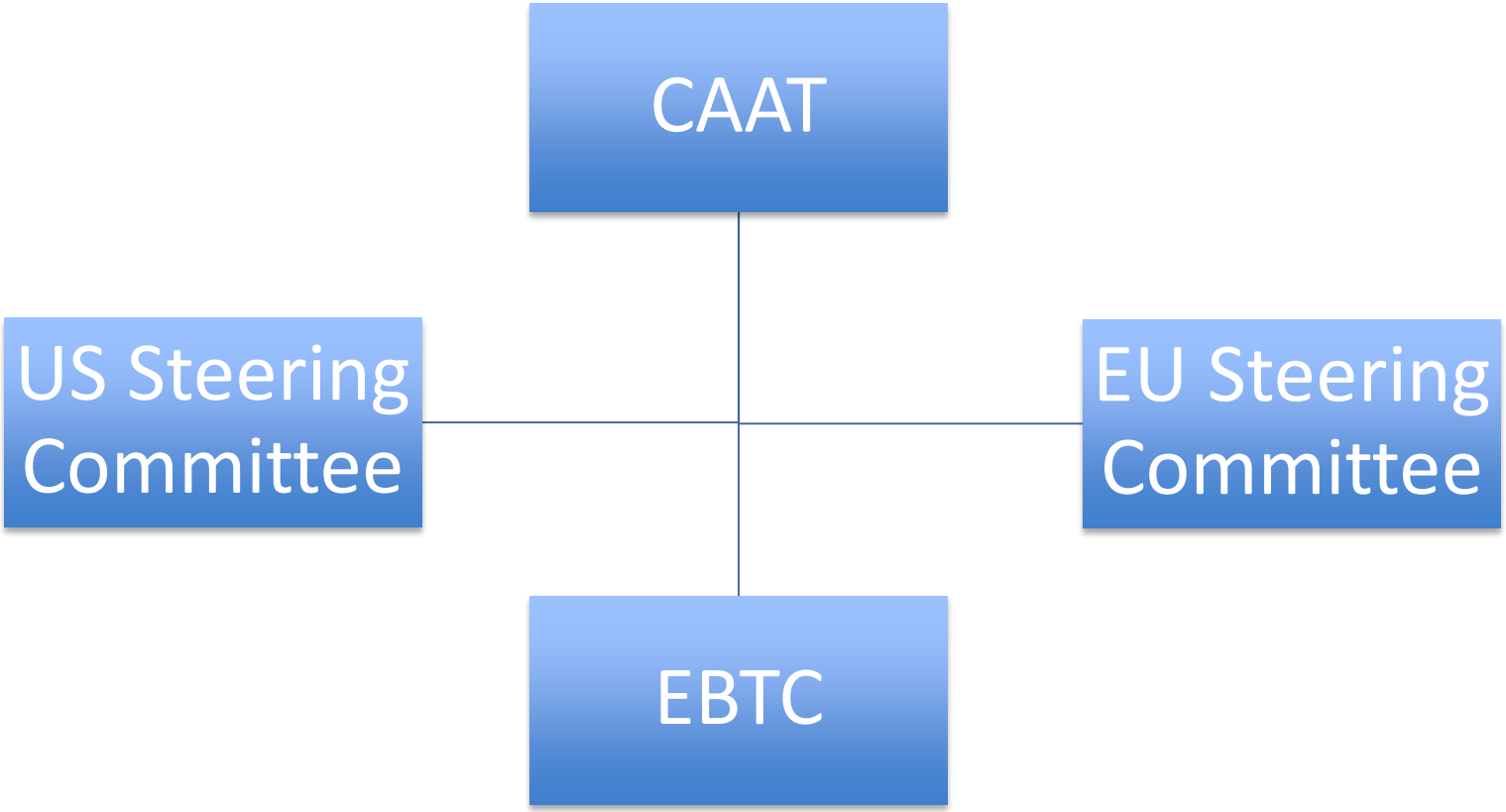
EBTC Funding:

Johns Hopkins School of Public Health / Private charitable foundation (93%)
ExxonMobil Foundation (7%)

Where is EBTC?



Original EBTC Structure

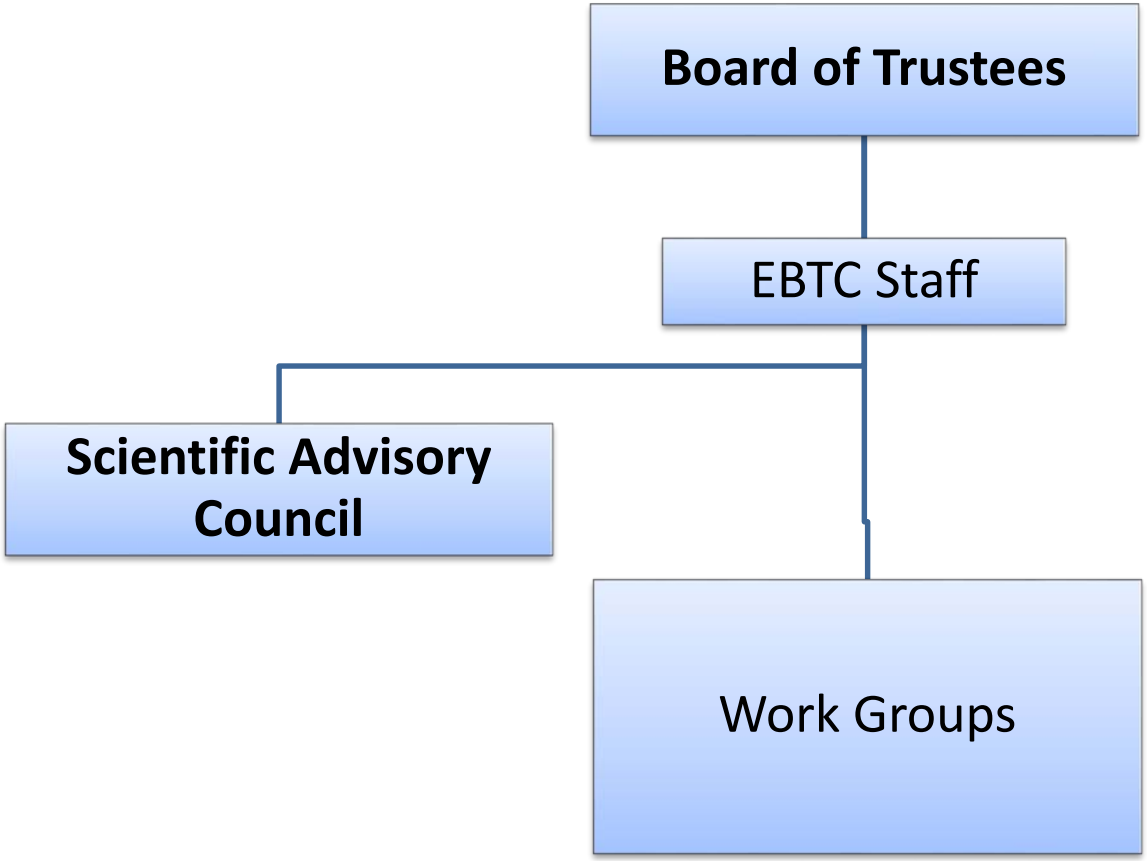


Why change the governance structure?

- To maximize:
  - Unification of EU and US steering in one governance structure
  - Balance of stakeholders from all sectors
  - Credibility
  - Transparency of all projects and management decisions
  - Inclusivity, flexibility
  - Free debate and clear decisions about issues that arise
- To minimize:
  - Potential for bias and conflict of Interest via balance of stakeholders on the Board and Scientific Advisory Council
  - Redundancies

New EBTC Structure

(est. May 2015 by The Governance Work Group)



Board of Trustees

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- Ian Kimber, Professor of Toxicology, University of Manchester
- Thomas Hartung, Chair, Evidence-Based Toxicology, Johns Hopkins University, CAAT
- Nancy Beck, Sr. Director, Regulatory Science Policy, American Chemistry Council
- James Freeman, Distinguished Toxicology Associate, ExxonMobil
- Thomas Singer, VP, Discovery, Roche
- Didier Verloo, Head of Assessment and methodological support unit, European Food safety Agency
- Sebastian Hoffmann, SEH Consulting (EBTC staff) *non-voting member*
- Martin Stephens, Johns Hopkins University (EBTC staff) *non-voting member*
- Katya Tsaioun, Johns Hopkins University (EBTC staff) *non-voting member*

Scientific Advisory Council

Name	Affiliation	Region	Sector
Fran Kruszewski	ACI	USA	I
Manoj Lalu	OHRI	Can	A
Julie Goodman	Gradient	USA	C
Didier Verloo	EFSA	EU	G
Vince Cogliano	EPA	USA	G
Carl Westmoreland	Unilever	UK	I
Suzanne Fitzpatrick	FDA	USA	G
Malcolm Macleod	CAMARADES	EU	A
Richard Judson	EPA	USA	G
Mel Andersen	Hamner	USA	N
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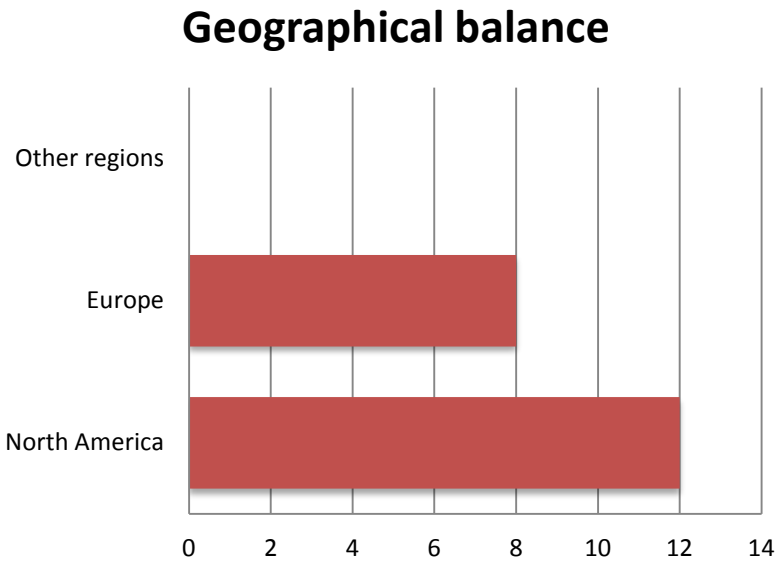
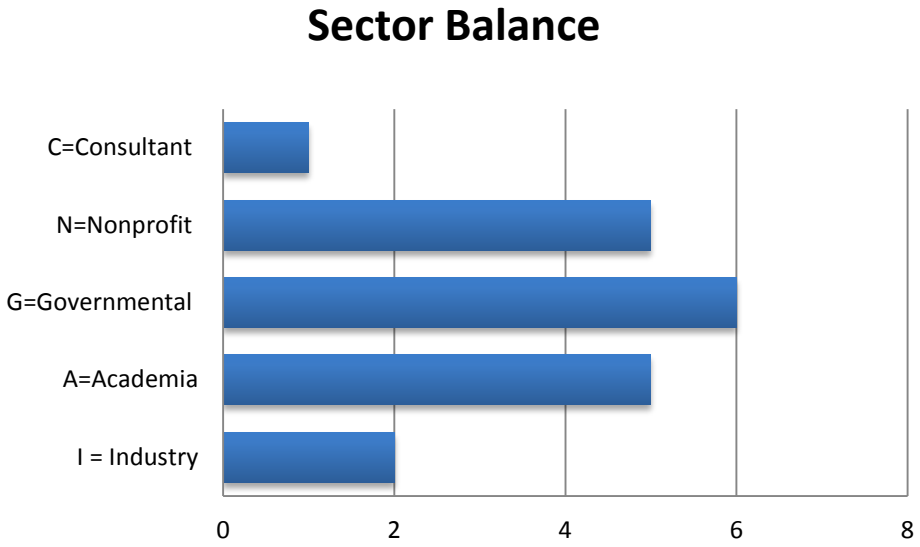
Barry Hardy	OpenTox	EU	N
Paul Whaley	Cancer Prevention and Education Society	EU	N
Hubert Dirven	Norwegian Institute of Public Health	EU	G

SAC Balance of Stakeholders





EBTC Work Groups

- Methodology WG
  - EBT Primer (in review now. S. Hoffmann)
  - Study quality paper (in revision, M. Stephens)
  - Emergence of SR in toxicology (draft, M. Stephens)
- Zebrafish embryotoxicity test WG
  - Zebrafish embryotoxicity test SR

Zebrafish Systematic Review

Zebrafish Work Group

Martin Stephens (EBTC Founding Director, Johns Hopkins Bloomberg School of Public Health, **EBT**)

Rob Wright (Johns Hopkins University, Informatics, **Literature search strategy**)

Sebastian Hoffmann (EBTC staff, Germany, **Systematic Reviews**)

Elizabeth Ghandakly, Esq. **Reviewer 2**

Alexandra Maertens (Post-Doctoral Fellow, EBTC, Johns Hopkins, **Reviewer 1**, **Informatics**)

Francois Busquet (Johns Hopkins Center for Alternatives to Animal Testing (CAAT), **Zebra Fish biology**)

Catherine Willett (Humane Society of the United States, **Zebra Fish Biology, Tests Validation**)

Burkhard Flick (BASF, **observer**)

Manoj Lalu (Ottawa Hospital Research Institute, **Epidemiology**)

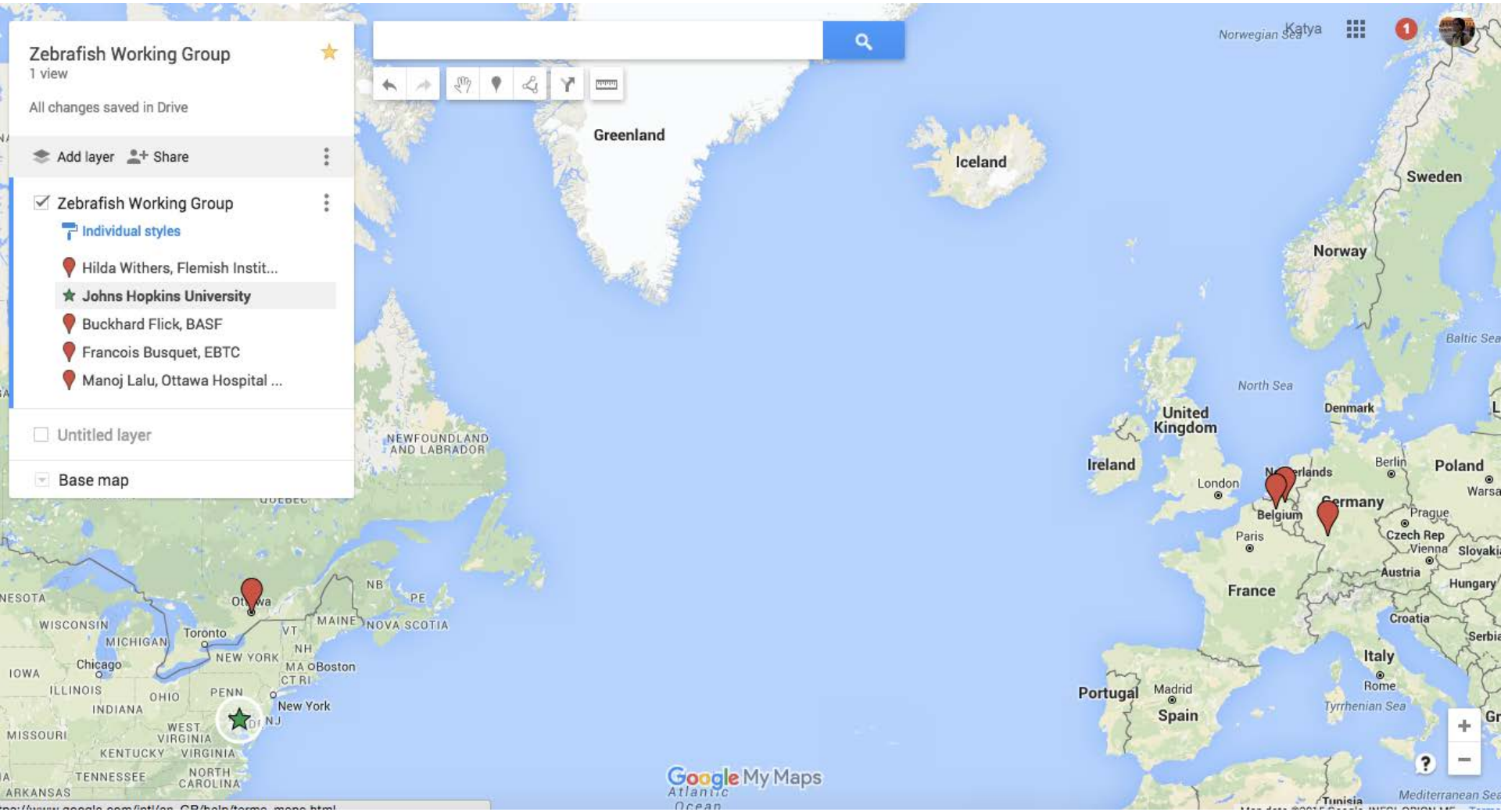
Hilda Witters (Flemish Institute for Technological Research, **Reviewer 3**, **Systematic Reviews**)

Kary Thompson (BMS, **observer**)

Katya Tsaioun (Johns Hopkins, ***Manage project***)

Thomas Hartung (Johns Hopkins, **EBT**)

Zebrafish Work Group



Drivers for the project

1. **Exploring Systematic Review (SR)**
   - Ensuring transparency, objectivity, consistency
   - Adapting Cochrane *Handbook for Diagnostic Test Accuracy Reviews* from medicine to toxicology
2. **Rethinking Validation**
   - Recognized disadvantages of historical approaches
   - How should studies on a test's performance be assessed?
   - Need approaches that are more transparent, objective, and structured
   - Potential insights to be gained from lessons learned in medicine
3. **Assessing Zebrafish Embryo Testing (ZET)**

ZET as a predictor (INDEX TEST) of teratogenesis in mammals (rats and rabbits) (COMPARATOR TEST) used in OECD TG 414

- Current use: Screening & prioritization
- Potential use: Refinement and (partial) replacement

Starting point:
Cochrane *Handbook for DTA Reviews*

http://srdta.cochrane.org/handbook-dta-reviews

"Systematic reviews of diagnostic test accuracy are very different from intervention reviews."

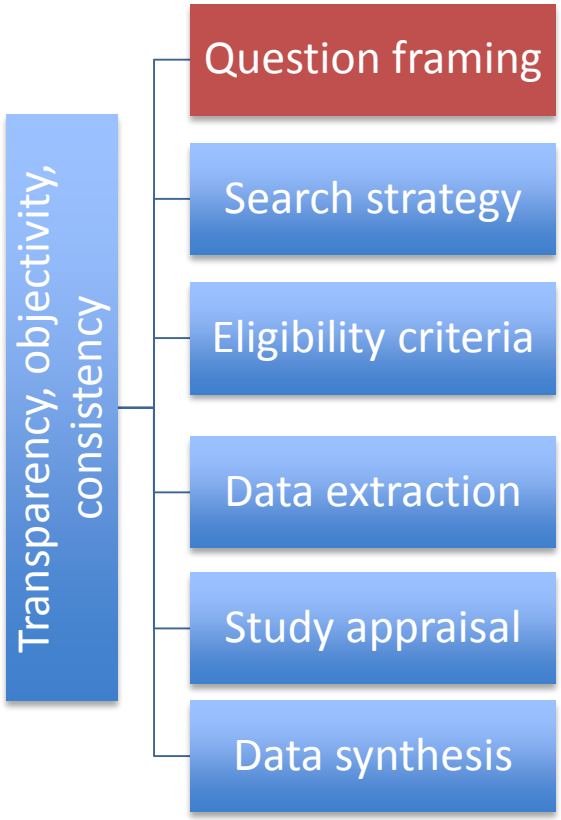
http://www.fraunhofer.de

http://www.nature.com

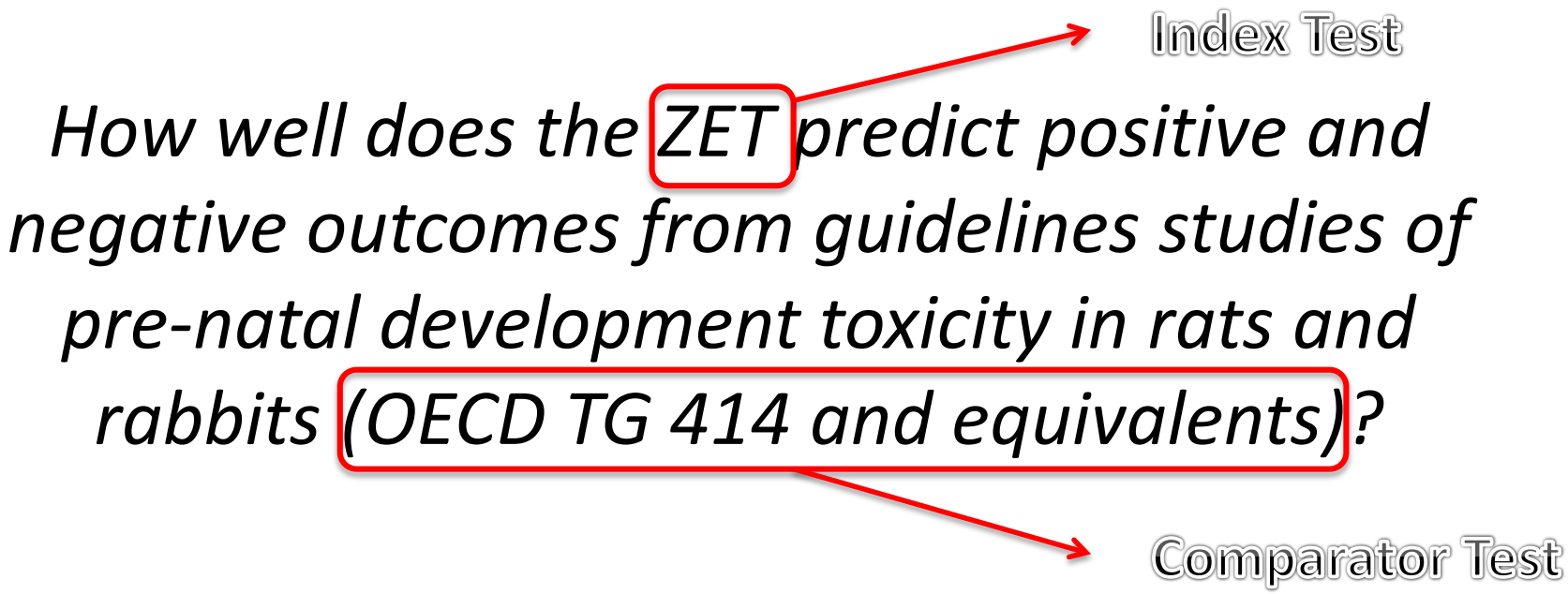
Medicine

Toxicology

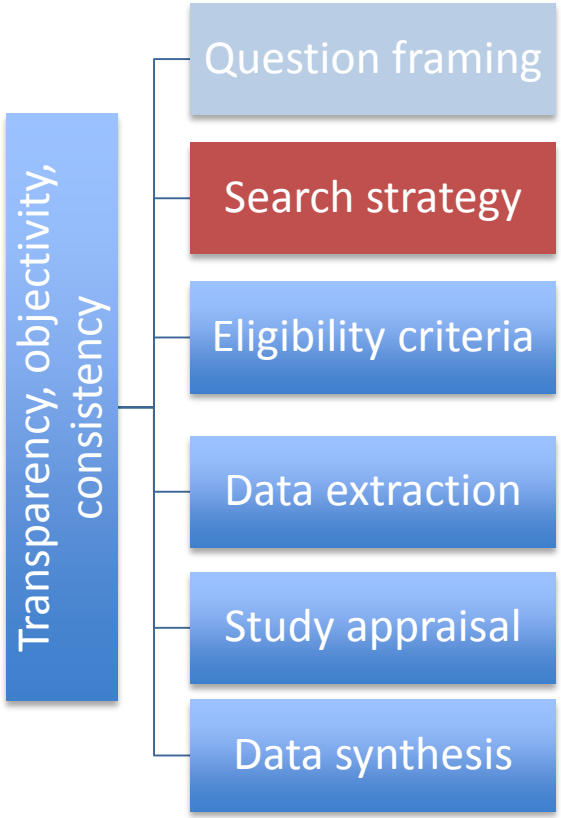
Steps in systematic review



Question Framing



Steps in systematic review



The SR protocol

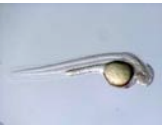
The Zebrafish Embryo Test as a Predictor of Mammalian Developmental Toxicity

A Draft Systematic Review **Protocol**

Draft of November 12, 2014

Table of Contents

Search strategy



INDEX

ZET literature search
Databases: PubMed, Biosis, Toxline, & Embase.
N = 6,327

# of additional records identified through other sources? N = 0

Search results combined; duplicates removed. N = 5,074

Title & abstract review & application of inclusion criteria. N = x

Included: N = x

Excluded: N = x

Full manuscript review and application of remaining inclusion criteria. N = x

Included: N = x

Excluded: N = x

Chemicals identified

COMPARATOR

Mammalian literature search
Databases: PubMed, Biosis, Toxline, & Embase

# of additional records identified through other sources?

Search results combined; duplicates removed (n = x)

Articles screened on basis of title and abstract

Included (n = xxx)

Excluded (n = x,xxx)
Not …: xx
Not …: xx
Different…:xx

Manuscript review and application of inclusion criteria

Included (n = xx)

Excluded (n = xxx)
Not …: xx
Not …: xx
Different…:xx

Data analysis

Question Framing, Protocol, Search Strategy & Eligibility Criteria

Question Framing:

How well does **ZET** predict the presence or absence of malformations in studies of pre-natal development toxicity in rats and rabbits (**OECD TG 414 and equivalents**)?

Search Strategy:

1. Relevant studies & chemicals first identified on Zebrafish
2. Search for same chemicals in mammalian studies.
3. Two independent researchers + information specialist.
4. Operationalize the process on a pilot study.

Drafting the protocol

>20 Eligibility Criteria:

- e.g. zebrafish studies with ≥10 eggs per conc. (**inclusion**) or studies on transgenic zebrafish (**exclusion**).

Operationalization of the process: a pilot

- A pilot study was suggested to help operationalize the process and refine protocol with random selection of 50 studies
- 5 compounds were studied in the pilot studies that met inclusion/exclusion criteria

Chemical
Albendazole
Ellagic acid
Estrogen
Ethanol
TCDD

Pilot study

INDEX

ZET literature search
Databases: PubMed, Biosis, Toxline, & Embase.
N = 11,741

# of additional records identified through other sources? N = 0

Search results combined; duplicates removed. N = 5,074

Studies **randomly selected** for title & abstract review & application of inclusion criteria. N = 50

Included: N = 14

Excluded: N = 36

Full manuscript review and application of remaining inclusion criteria. N = 14

Included: N = 5

Excluded: N = 9

5 chemicals identified

Mammalian literature search
Databases: ECHA, HSDB, DART

# of additional records identified through other sources?

Search results combined; duplicates removed (n = 168)

Articles screened on basis of title and abstract

Included (n = 17)

Excluded (n = x,xxx)
Not …: xx
Not …: xx
Different…:xx

Manuscript review and application of inclusion criteria

Included (n = 17)

Excluded (n = xxx)
Not …: xx
Not …: xx
Different…:xx

Data Analysis

COMPARATOR

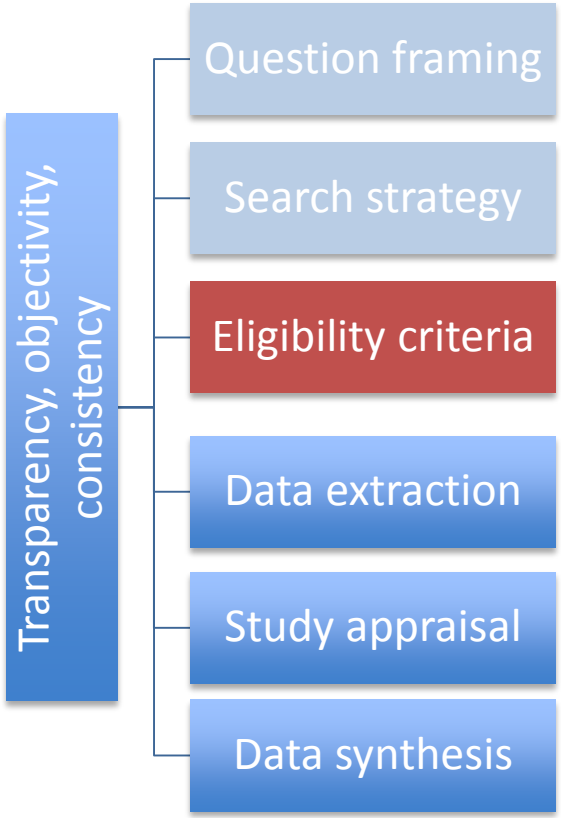
Search Strategy: Problems

- Results took a long time (to complete the search and to de-dupe):
  - Heterogeneity of studies
  - Lack of details in reporting
  - Lack of abstract structure
- Several chemicals were contributing to the problem
  - Ethanol – large number of studies were looking at neurodevelopmental endpoints and were not relevant for our search
  - Any abstract that mentioned TCDD in the context of developmental toxicity was included
  - Some chemicals (e.g. albendazole) are not widely studied; did not appear in the primary literature but searching HSDB pointed to a WHO/FAO hazard assessment which referenced several guideline compliant studies

Pilot Study: Mammalian Search Strategy

- Wide literature search for OECD 414 mammalian studies of the 5 chemicals resulted in 11,000 studies
- Since the mammalian tests are the comparator test, and the question about OECD guideline tests, regulatory databases were searched for the pilot:
  - ECHA (European Chemical Agency) -> Regulatory studies
  - HSDB (Hazardous Substance Database) -> Curated by chemical
  - DART (Developmental and Reproductive Toxicity Database) -> Indexed by chemical; curated
- Mammalian search strategy using this methodology took approximately 1.5 months to gather and enter the data

Steps in systematic review



Eligibility Criteria

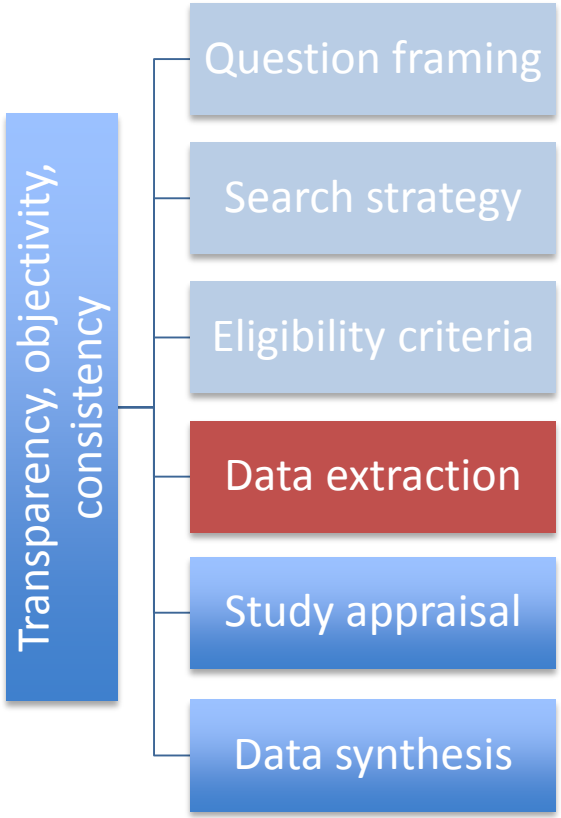
Inclusion Criteria for Mammalian Studies

- Studies investigating developmental toxicity endpoints
- Studies conducted on rats or rabbits in which the species' strain is reported
- Studies reporting original data
- Studies in which doses are administered orally via gavage or in food
- Studies in which the endpoints associated with positive findings are documented
- Only studies that had either explicitly stated they were doing a guideline compliant (with either minor deviations/enhancements) were included

http://www.psu.edu

http://www.scientificamerican.com

Steps in systematic review



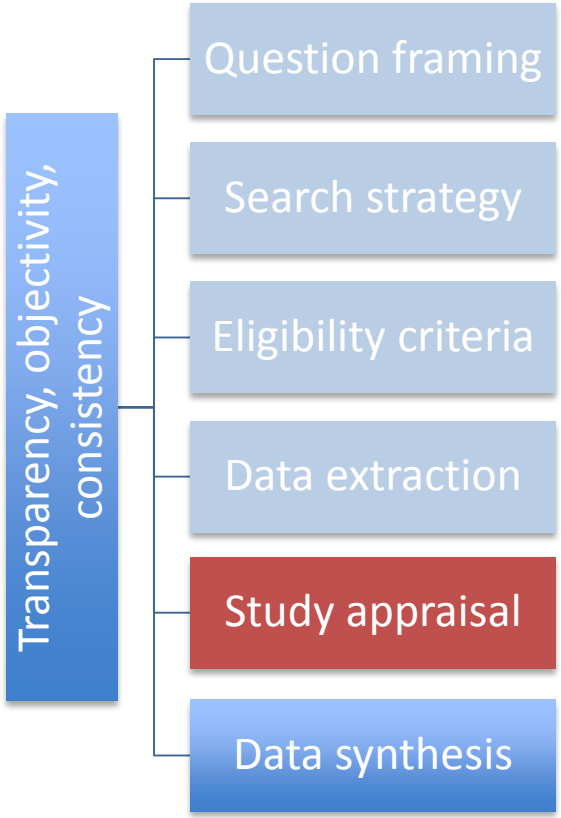
Data extraction

Data extraction: two independent reviewers are examining studies and extracting data.

Data Extraction Table

Source	Chemicals assessed	# Zebrafish embryos per dose group	Age at first exposure (HPF)	Exposure duration (HPF)	Chemical concentrations used	Controls (-, +, solvent)	Water temp. (degrees C)	Water pH	Dechorionation?

Steps in systematic review



Appraisal of Methodological Quality

- Random allocation of treatment
- Allocation concealment
- Blinding of research personnel
- Attrition rates low and similar across groups
- Blinding of outcome assessors
- Selection of appropriate control groups
- All measured outcomes reported
- Every animal accounted for
- Sample size calculation
- Statistical model explained
- Test animal details
- Optimal time window used
- Conflict of interest disclosed

Within the primary published literature, very few studies explicitly addressed *any* of these criteria

Lessons learned

Reporting quality:

- Zebrafish species frequently not reported (hence, transgenic species exclusion criteria could not be applied)
- Chemicals names are not consistently reported
- Not sufficiently quantified and differentiated the reported endpoint criteria (e.g., it was sometimes difficult to determine whether report of death was the embryo or the fish).
- Video surveillance reporting criteria not standardized

Lessons learned and opportunities

There are no clear methodologies for EBT, particularly for test methods performance

- EBTC formed a Methods Work Group, has written an EBT Primer and is seeking to build on this work in a partnership

Is PROSPERO adaptable enough to publish EBT protocols?

- Need a common portal for publishing protocols

There are no toxicology-friendly shared data extraction tables adapted for toxicology studies

- Data extraction tools for toxicology studies and test methods are needed

Conclusions

- Novel application of SR for developmental toxicology
- Pioneering new approach to assessing test method performance/validation
- Written protocol allowed us to make translation from medicine to toxicology
- Pilot study allowed us to operationalize this process
- Limitations (e.g., focusing on malformations only and no human data to serve as independent standard)
- Lessons learned and pilot results to be published (Q1 2016) and definitive study completed (Q2 2016)

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Evidence-based Toxicology Collaboration

Newsletter

No. 1, 2012

We are pleased to present the first issue of the newsletter of the Evidence-Based Toxicology Collaboration (EBTC).

The Evidence-based Toxicology Collaboration has been established to promote the use of evidence-based approaches in toxicology and related safety sciences. Such approaches are guided by the themes of transparency, objectivity, and consistency. The anticipated benefits of an evidence-based toxicology

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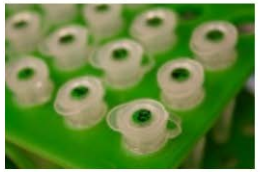

Steering Committees

EBTC at conferences

SOT 2016 Workshop

Paradigm change in toxicology: what will it take to bring advances in the science of toxicology into regulatory use?

Chairs: Katya Tsaioun, EBTC and John-Michael Sauer, Critical Path Institute

40th Annual Winter Meeting of The Toxicology Forum February 8-10, Washington, DC.

ICT

Session on Evidence-based Toxicology accepted for 2016

Other activities

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]

http://www.ebtox.com

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Evidence-based Toxicology Collaboration

Mission

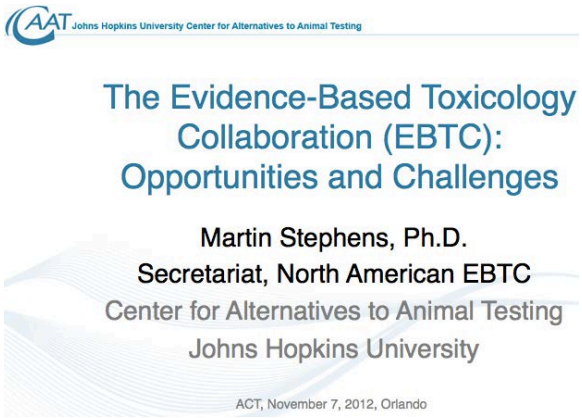
Vision

The EBT Collaboration has closely coordinated steering committees in North America and Europe, with members drawn from academia, government agencies, and industry.

Current and planned EBTC activities:

- Furthering the conceptual development of EBT
- Producing guidance on EB approaches
- Fostering case studies of EB applications to toxicology
- Educating interested scientists about EBT approaches
- Advocating the use of evidence-based methods in safety sciences
- Keeping stakeholders up to date on our activities

Subscribe to our newsletter—visit http://www.ebtox.com



Johns Hopkins University Center for Alternatives to Animal Testing

The Evidence-Based Toxicology Collaboration (EBTC): Opportunities and Challenges

Martin Stephens, Ph.D.

Secretariat, North American EBTC

Center for Alternatives to Animal Testing

Johns Hopkins University

ACT, November 7, 2012, Orlando

Perspectives | Correspondence

All EHP content is accessible to individuals with disabilities. Fully accessible (Section 508–compliant) HTML versions of these articles are available at http://dx.doi.org/10.1289/ehp.1307727 and http://dx.doi.org/10.1289/ehp.1307727R.

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Instruments for Assessing Risk of Bias and Other Methodological Criteria of Animal Studies: Omission of Well-Established Methods

http://dx.doi.org/10.1289/ehp.1307727

In response to the systematic review by Krauth et al. (2013) of instruments for assessing animal toxicology studies for risk of bias and other aspects of quality, we propose the need for a broader perspective when appraising—and hopefully improving—

These additional publications describe design, conduct, and reporting criteria that form the basis of the methodologies employed globally to assure quality and reliability of *in vitro* toxicological investigations for regulatory assessment of human and ecological health hazards. Because the application of systematic review and related evidence-based approaches in toxicology is still in its infancy, it is especially important at this time to recognize the contributions of these publications.

The omission of these publications by

S. Hoffmann, J.R. Fowle III, and J. Goodman are consultants and have worked on a range of toxicity and risk assessment issues for a wide variety of clients. R.A. Becker and N.B. Beck are employed by the American Chemistry Council, a trade association of chemical manufacturers. A. Boobis, D. Fergusson, M. Lalu, and M. Leist are employed by institutes of higher education. In the past 3 years, A. Boobis and M. Leist have worked on a range of toxicity and risk assessment issues for a number of clients; this has included some consultancies.

All authors contributed equally and are listed in alphabetical order.

Nancy B. Beck,[1] Richard A. Becker,[1]* Alan Boobis,[2]* Dean Fergusson,[3]* John R. Fowle III,[4]* Julie Goodman,[5]* Sebastian Hoffmann,[5]* Manoj Lalu,[3]* Marcel Leist,[6]* and Martin L. Stephens[5]*

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Thank you!

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