Bioaccumulation Technical Committee

John Nichols, PhD
USEPA

HESI Annual Meeting
Washington, DC

10 June 2014

ILSI Health and
Environmental Sciences
Institute

HESI Bioaccumulation by the Numbers…..

35+
Partner organizations

9
years of improving the science of 'B' assessment

7
International workshops

2
Hands-on in vitro method training courses

23
Presentations at international meetings since 2009

19
Peer-reviewed publications (and counting)

9
FUNDED RESEARCH PROJECTS

Committee Mission and Objectives

- Develop the tools needed for assessing the potential bioaccumulation of organic chemicals
- Address how the various metrics used to assess bioaccumulation can be integrated to develop an overall weight-of-evidence approach for deriving assessment conclusions
- Partner with other groups involved in the advancement of bioaccumulation methods and assessment.

Regulatory needs for bioaccumulation assessment

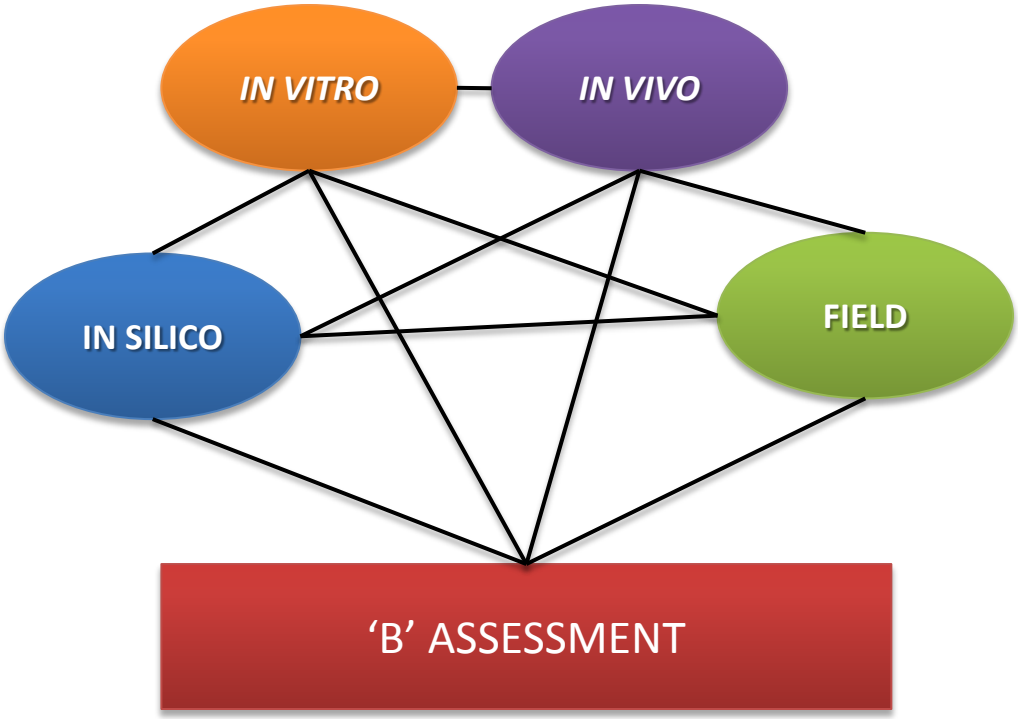
- The UNEP global Stockholm Convention on persistent organic pollutants, effective in 2004, led to an increased need for assessment of chemicals that are persistent (P), bioaccumulative (B), and inherently toxic (iT)

- Bioaccumulation data are scarce (<3% of chemicals with data)
  - September 2006, Canada, Domestic Substance List: >96% of initial categorization decisions on the bioaccumulation potential of organic chemicals (~10,000) based on model predictions[1]
  - 2006 – 2012, Europe, REACH legislation: It is anticipated that ~3,025 chemicals will require some form of bioaccumulation testing[2]

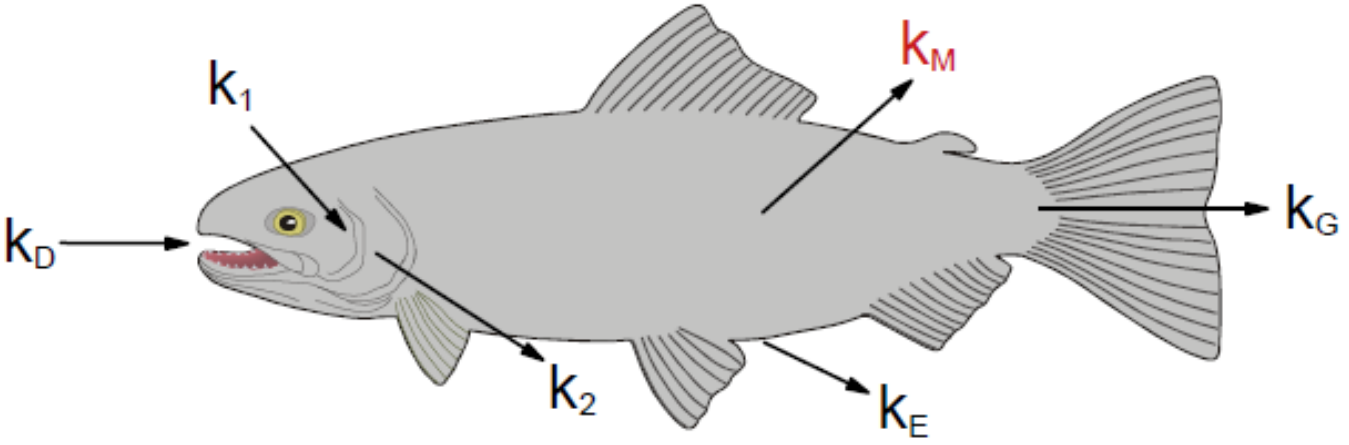
[1]Arnot and Gobas, 2006

[2] ECETOC 2005. Reducing Animal Use in Chemical Management for Environmental Safety

Weight-of-evidence approach for ‘B’ assessment



Background



$$C_{FISH} = (k_1 C_W + k_D \Sigma P_i C_{D,L})/(k_2 + k_E + k_M + k_G)$$

Arnot and Gobas, 2003

Bioconcentration factor (BCF)

- Defined as the steady-state chemical concentration in a fish divided by that in the water, assuming a water-only exposure

$$BCF = C_{FISH}/C_{WATER}$$

- Widely used by regulators as a surrogate for the things we would really like to know (e.g., bioaccumulation by all routes, biomagnification, trophic magnification)

For a water-only exposure, ignoring growth

$$C_{FISH} = k_1 C_W / (k_2 + k_E + k_{MET})$$

And, since $BCF = C_{FISH} / C_W$

$$BCF = k_1 / (k_2 + k_E + k_{MET})$$

Importantly, k_1, k_2, and k_E can be predicted for many compounds using log K_{OW}-based relationships. Our challenge, therefore, is to estimate k_{MET}

How do we predict metabolism impacts on BCF?

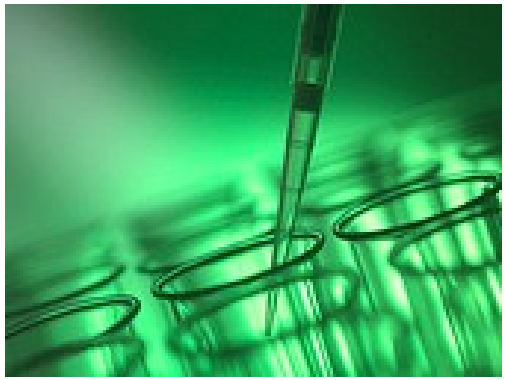
- One approach: Collect in vitro metabolism data and extrapolate to the whole animal

  - Builds on methods developed by the pharmaceutical industry for pre-clinical screening of drug candidates.

  - Based on the principle of intrinsic hepatic clearance which can be thought of as enzymatic activity under non-saturating conditions (i.e., $CL_{INT,IN\ VITRO} \approx V_{max}/K_m$)

  - Employs scaling factors and a physiological liver model to translate $CL_{INT,\ IN\ VITRO}$ into an estimate of blood flow cleared of chemical per unit time – i.e., the "hepatic clearance" (CL_H; L/h or L/h/kg).

  - CL_H is then translated into an estimate of whole-body metabolism rate (k_{MET}) which becomes an input to standard bioaccumulation models

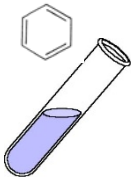


Overview of in vitro methodology

Analyze

Extract parent compound

Quench reaction at regular intervals



Incubate w/ test chemical

Isolate liver S9 fraction or hepatocytes

Calculate in vitro intrinsic clearance rate for the parent chemical,

- Liver weight (g/kg)
- Hepatocellularity (cells/g liver)
- S9 protein content (protein/g liver)

Calculate in vivo intrinsic clearance rate (L/h/kg)

- Liver blood flow rate (L/d/kg)
- Binding corrections as appropriate

Calculate hepatic clearance (L/h/kg)

- Apparent volume of distribution (L/kg)

Calculate of whole-fish metabolism rate constant k_{MET} (1/d)

Combine k_{MET} with estimates of k_1, k_2 and k_E to simulate C_{Fish} and predict BCF

Nichols et al., 2013; Cowan-Ellsberry et al., 2008; Han et al., 2007

Does it work?

- To date, several groups have extrapolated *in vitro* metabolism data for fish to the intact animal and used this information as an input to models of chemical bioconcentration

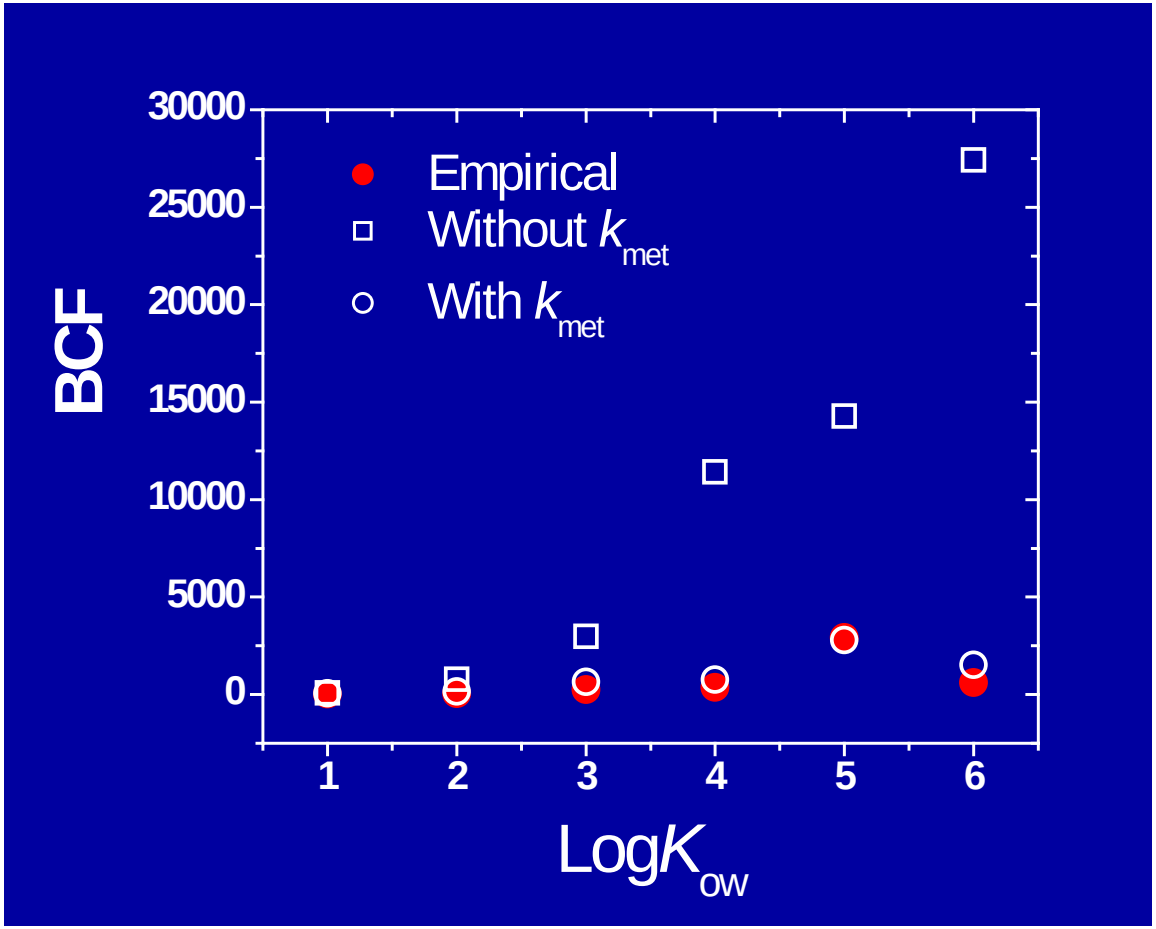
- These “proof of concept” studies show that incorporating *in vitro* metabolism data into the models substantially “improves” predicted BCF values (compared to predictions without metabolism) by moving them in the direction of measured values[1]

- The development of this method has caught the attention of regulatory agencies

- Companies have become early adopters of these methods and have begun using them to support their regulatory submissions

[1]Han et al., 2007, 2009; Cowan-Ellsberry et al., 2008; Dyer et al., 2008; Gomez et al., 2010

Potential of the method – hepatocyte results[1]



[1]Data from Han et al., 2007

Potential of the method – S9 results[1]

Substance	Log K_{ow}	**Predicted BCF** (using standard QSARs)	***In vitro* intrinsic clearance rate** (ml/h/mg protein)	**Refined BCF estimates (fu =1.0)**	***In vivo* BCF** *(OECD 305)*
Chemical A	4.0	202 - 1034	0.89	105	**295-317**
Chemical B	4.2	274 - 1607	1.18	119	**31-310**
Chemical C	4.7	90 - 7882	3.50	148	**600-900**
Chemical D	4.9	794 - 6624	0.07	776	**381**
Chemical E	5.3	1460 - 12370	~0.15	599	**85-137**
Chemical F	5.65	2678 - 18370	0.35	406	**500**
Chemical G	5.7	2680 - 18370	~0.02	3827	**867-3920**
Chemical H	6.3	2456 - 20420	2.39	189	Not determined

[1]Data for eight fragrance molecules presented with permission of Heike Laue. The paper that describes this work has been submitted.

Promising method – what next?

- Ultimate goal: regulatory acceptance
  - Quotation from REACH guideline. *"In vitro methods have the potential to provide important data on bioaccumulation assessments…These methods may become an important part of future test strategies, but their applicability is currently limited due to the lack of standardized protocols, limited validation based on small data sets. Further evaluation work is necessary before they can be recommended for use within an ITS."*
- Needs
  - Standardized method ("reliability")
  - Robust dataset to demonstrate the accuracy of in vitro – in vivo extrapolations ("relevance")
  - Multi-stakeholder input ("implementation")

Parallel ring-trial

- Objective: Further evaluate in vitro methods (trout S9 and hepatocytes) used to assess chemical biotransformation in fish in order to support OECD TG development.

- Aims:
  - Compare in vitro assay performance across participating laboratories
  - Provide recommendations for future use of these methods in bioaccumulation assessments
  - Provide data that can be used to validate modeled bioaccumulation predictions (i.e., a link to planned in vivo BCF testing efforts)
  - Provide information required to develop an OECD TG for these methods

Parallel ring-trial – progress to date

- A Standard Project Submission Form (SPSF) was submitted to the OECD Validation Management Group for Ecotoxicity testing (ECO VMG) in 2013. The lead countries are the U.S. and European Commission
- As of April 2014, the HESI ring trial is now part of the official OECD Test Guidelines Programme Workplan
- Partners and test chemicals being identified (currently 9 laboratories)
- Biological material has been prepared
- Laboratory work to initiate summer 2014

Summary

- The HESI Bioaccumulation Project Committee continues to support focused research on topics ranging from development of computational models to collection and interpretation of field data

- Work on the development and validation of in vitro biotransformation test methods has progressed to the point that we are moving toward of establishment of OECD Test Guideline

- Research to support development and approval of an OECD TG is ongoing.

Acknowledgements

Leadership & Steering Team

- Jean Domoradzki, Co-Chair, Dow Corning
- Mark Bonnell, Environment Canada
- Lawrence Burkhard, US EPA
- Marlies Halder, EC JRC
- Xing Han, DuPont
- Duane Huggett, U North Texas
- Thomas Parkerton, ExxonMobil
- Caren Rauert, UBA

- HESI manager: Michelle Embry
- HESI associate: Connie Chan-Le

Acknowledgements

Core Committee Participants – Academic & Government

- Aarhus University
- Arnot Research & Consulting
- CERI
- ENVIRON
- Environment Canada
- European Commission, Joint Research Center
- Federal Environment Agency (UBA, Germany)
- KJohanning Consultancy
- Michigan State University
- Norwegian Institute for Water Research (NIVA)
- Pacific Northwest National Laboratories
- Roskilde University
- Simon Fraser University
- Swiss Federal Institute of Aquatic Science and Technology (Eawag)
- UK Environment Agency
- University of Bern
- University of North Texas
- University of Queensland
- University of Stockholm
- University of Toronto
- University of Windsor
- US Environmental Protection Agency
- VU University Amsterdam
- Wageningen University
- Waterborne Environmental

Acknowledgements

Committee Sponsors

- AstraZeneca AB
- The Dow Chemical Company
- Dow Corning Corporation
- DuPont
- ExxonMobil
- L'Oréal
- Pfizer
- The Procter & Gamble Company
- Research Institute for Fragrance Materials (RIFM)
- SC Johnson