ILSI Health and Environmental Sciences Institute

In-vivo bioaccumulation experts workshop

In Vivo Testing of Chemicals that Undergo Substantial Biotransformation: An Opportunity to Advance In Vitro-In Vivo Metabolism Extrapolation Procedures for Fish

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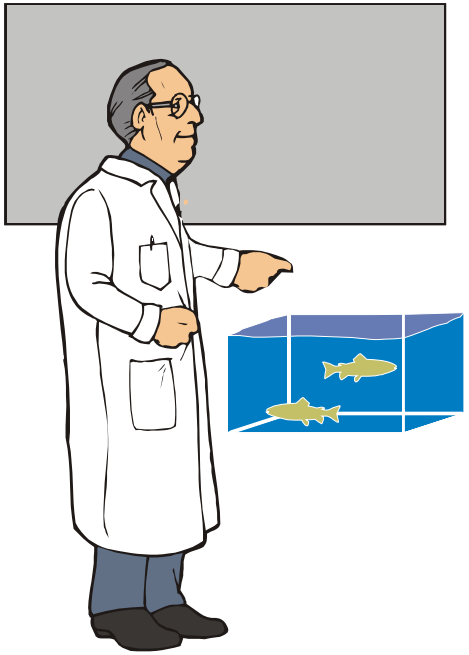
Background

- Mathematical models consisting largely of K_{OW}-based relationships accurately predict measured levels of accumulation for many compounds, provided they don't undergo substantial metabolism.
- Metabolism tends to reduce bioaccumulation. Unfortunately, it does not correlate simply with chemical K_{OW}.
- For this reason, metabolism is the most critical uncertainty in many bioaccumulation assessments for fish.



How do we predict metabolism impacts on BCF?

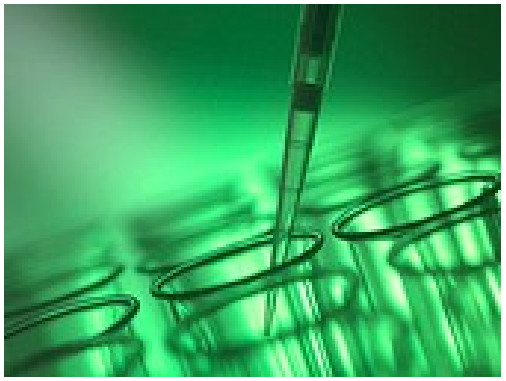
- One approach: Collect in vitro metabolism data and extrapolate to the whole animal

  - 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_{METAB}) which becomes an input to standard bioaccumulation models



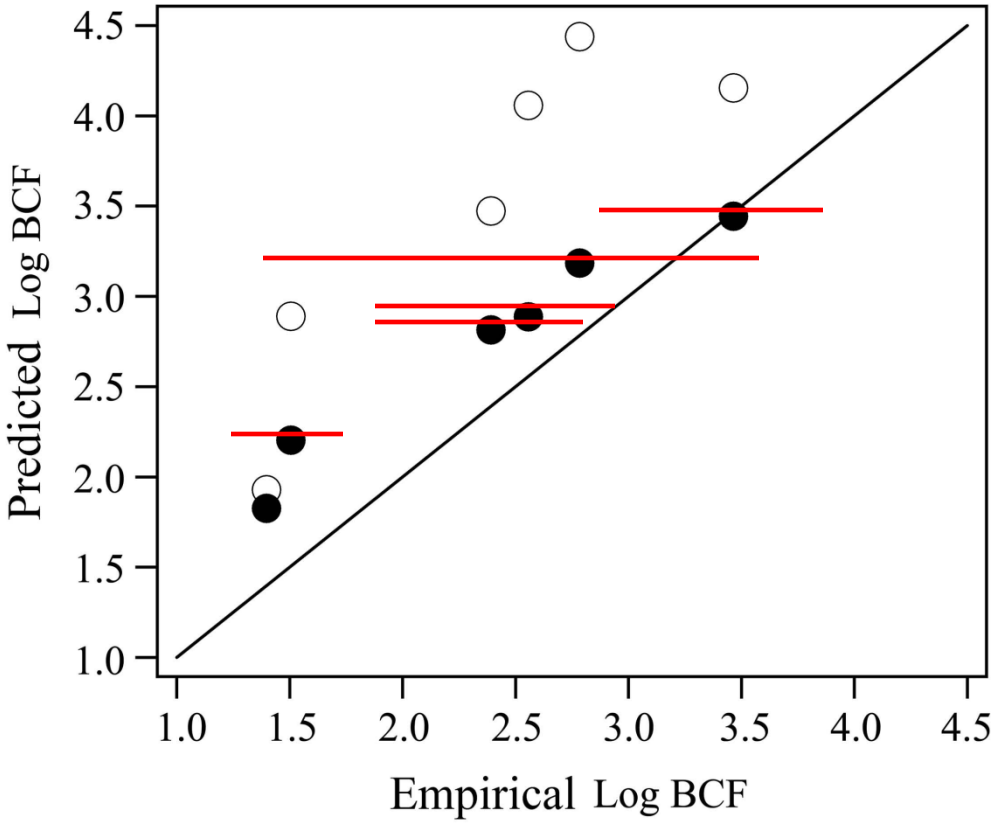
But does it work?

- 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]

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

But does it work?



From Nichols et al., 2009, Integ. Environ. Assess. Manage., 5:577-597. Data are from Han et al., 2007, Environ. Sci. Technol., 41:3269-3276

- ○ w/out in vitro data
- ● w/ in vitro data

But, predicted BCFs are based on in vitro data from trout while measured BCFs are based on data from bass, carp, fathead minnows, orfe, sheepshead minnows, salmon, bluegill sunfish, brook trout, brown trout, and rainbow trout

Ranges in four of the measured BCF values approach a factor of 10. For one compound (BaP) the range is > 100.

Other studies

Chemical	Predicted BCF (no metabolism)	Predicted BCF (with metabolism)	Measured BCF
Zoxamide (3.8)	618	155	400
Chlorpyrifos (4.7)	4288	873	1400

Cowan-Ellsberry et al., 2008, Chemosphere 70:1804-1817

Predicted BCFs are based on extrapolated data from rainbow trout while measured BCFs are from bluegill sunfish

Our work (unpublished)

Chemical	Predicted BCF (no metabolism)	Predicted BCF (with metabolism)	Measured BCF
Pyrene (4.88)	2209	423	940

In vitro and in vivo data are from strain- and temperature-matched rainbow trout.

However, trout used for in vitro testing were substantially larger than those used for BCF testing (500 g vs. 3 g). Is it possible that intrinsic clearance rates are slower in young animals?

Recommendations on future work / priorities

- Short term priorities
  - Standardization of in vitro test methods
  - Evaluation of different competing in vitro test systems (S9 vs. hepatocytes)

- Longer term needs
  - Refinement of several key model inputs (e.g., f_U and V_D)
  - Apply these methods to other fish species
  - Validation of these procedures by conducting experiments that “match” sources of in vitro and in vivo information (species, lifestage, temperature, etc.).
  - Validation of these procedures by conducting experiments that “marry” top down and bottom up k_{MET} estimation methods