Modeled effects of metabolism on chemical bioaccumulation in fish

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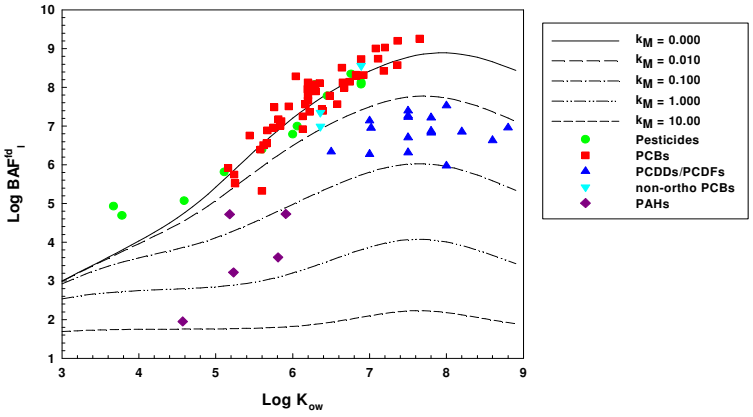
Focus on bioaccumulation

- Long-standing interest in legacy chemicals; PCBs, TCDD, PAHs, DDT/DDE
- Continuing effort to identify “new” contaminants that accumulate in humans and the environment; PBDEs, PFOS/PFOA
- Legislated evaluation of large chemical databases for bioaccumulation potential (as well as persistence and toxicity)
  - Canadian Domestic Substances List; approx. 20,000 compounds
  - REACH; approx. 100,000 compounds

Potential impact of metabolism on bioaccumulation



In vivo metabolism rates for fish

Substrate	Log K_{OW}	Species	k_M	Notes	Ref.
aniline	0.915	Medaka	5.52	o	8
4-chloroaniline	1.908	Medaka	5.04	o	8
2,3,4-trichloroaniline	3.203	Guppy	4.08	p	29
2,4,5-trichloroaniline	3.323	Guppy	3.12	p	29
benzo(a)anthracene	5.664	Fathead minnow	1.33	p	30
2,8-dichloro-dibenzo-p-dioxin	6.115	Goldfish	0.35	q	31
2,2′,4,6,6′-pentachloro-biphenyl	6.595	Guppy	0.015	p	32
1,2,3,7-tetrachloro-dibenzo-p-dioxin	7.307	Rainbow trout	0.096	q	33

o. Model estimated from product data
p. Model estimated from elimination rate and chemical K_{OW}
q. Estimated from BCF ratios in untreated and PBO treated groups;

In vitro metabolism rates for fish

Species, description	System	Substrate, conc.	Products	Accl. temp.	Assay temp.	V_{MAX}	K_M	Ref.
Medaka, adult, mix, 200-500mg,	Micr	aniline, 200-4000	phenylhydroxylamine	25 ^{0}C	25 ^{0}C	26.4	820	8
Medaka, adult, male, 200-500mg	Micr	4-chloroaniline, 200-4000	4-chlorophenylhydroxyl-amine	25 ^{0}C	25 ^{0}C	70.4	760	8
Medaka, adult, female, 200-500mg	Micr	4-chloroaniline, 200-4000	4-chlorophenylhydroxyl-amine	25 ^{0}C	25 ^{0}C	65.7	1990	8
Medaka, 10-12 month	Micr	trichloroethylene, 0-4000	chloral hydrate	NR	25 ^{0}C	213	540	9
Rainbow trout, imm 100-200g	Micr	benzo(a)pyrene, 2.5-120	all	10 ^{0}C	14 ^{0}C	60	64	10
Rainbow trout, 100-300g	Micr	4-chloroaniline, 200-4000	4-chlorophenylhydroxyl-amine	11 ^{0}C	11 ^{0}C	6.4	500	11
Rainbow trout, 100-250g	Micr	phenol, 1000-60000	hydroquinone	11 ^{0}C	11 ^{0}C	575	15000	12
Rainbow trout, 100-250g	Micr	phenol, 1000-60000	catechol	11 ^{0}C	11 ^{0}C	179	12000	12

Mammalian in vitro-in vivo metabolism extrapolations

- Methods developed primarily to facilitate the evaluation of drug candidates (high clearance compounds are dropped from further consideration)
- Based on the principle of intrinsic hepatic clearance ($CL_{in\ vitro,int} = V_{max}/K_m$)
- Employs scaling factors and a physiological liver model to translate $CL_{in\ vitro,int}$ into an estimate of blood flow cleared of chemical per unit time
- Supported by the recent development of “high throughput” methods for estimating K_m, V_{max} (substrate depletion)

Venous equilibrium liver model[a]

$$CL_h = Q_h f_u CL_{in\ vivo,int} / (Q_h + f_u CL_{in\ vivo,int})$$

where,

CL_h = hepatic clearance of blood (L/d/kg)
Q_h = hepatic blood flow (L/d/kg)
f_u = free fraction of chemical in blood (unitless)
$CL_{in\ vivo,int}$ = intrinsic *in vivo* clearance obtained by scaling *in vitro* metabolism data to the whole liver (L/d/kg)

a. Rowland et al., 1973; Wilkinson and Shand 1975

Characterize *in vitro* metabolism under linear conditions
(V_{max} = 1000 pmoles/min/mg protein, K_m = 100 pmoles/µl)

1

Calculate $CL_{in\ vitro,int}$ from the ratio V_{max}/K_m
(10 µl/min/mg microsomal protein)

2

Apply extrapolation factors to calculate $CL_{in\ vivo,int}$
(6500 µl/min/kg fish, or 9.36 L/d/kg fish)

3

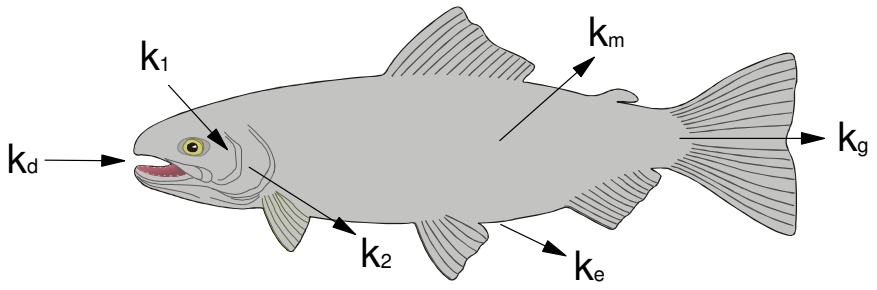
Apply well-stirred liver model to calculate CL_h
(1.79 L/d/kg fish)

4

Calculate whole-fish metabolism rate constant k_b
(0.035/d; corresponding to a metabolism t ½ of 19.8 days)

5

Combine with estimates of k_u and k_{nb} to simulate C_{fish} and predict the BAF

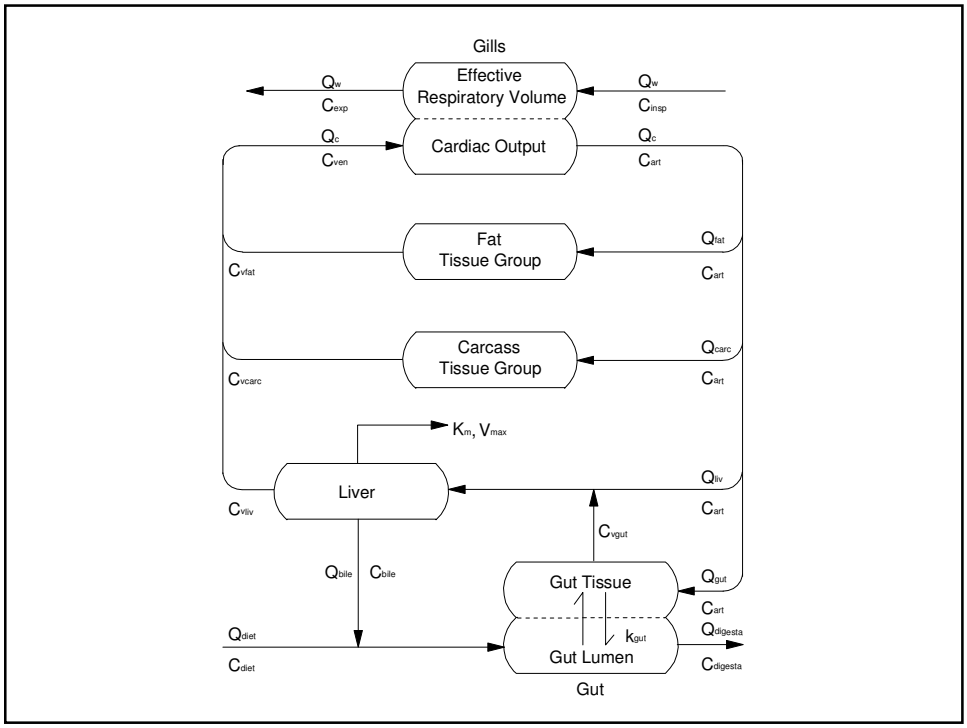


$$C_{fish} = (k_1 C_w + k_d \Sigma P_i C_{d,l})/(k_2 + k_e + k_m + k_g)$$

Arnot and Gobas (2004)

One-compartment bioaccumulation model

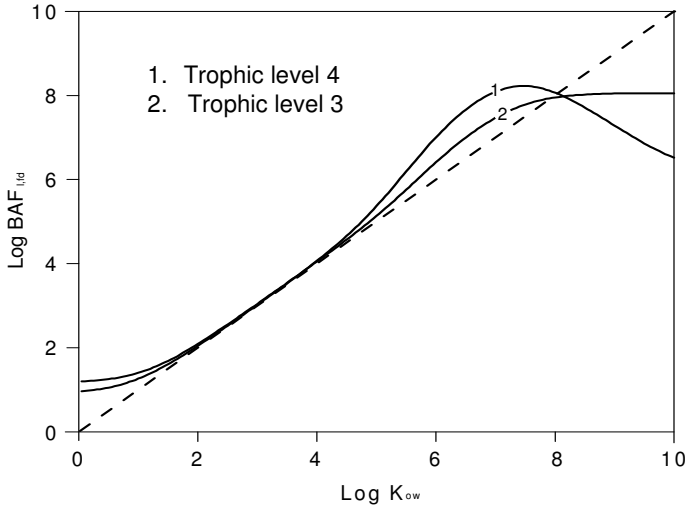
- Adapted from that used in the Arnot and Gobas (2004) food web model; adaptable to a wide range of conditions
- Predator - 10% lipid, 10% non-lipid organic matter, starting wt. of 1 kg
- Prey - 5.5% lipid, 14.5% non-lipid organic matter, starting wt. of 2.5 g
- Zooplankton - 2% lipid, 20% non-lipid organic matter
- Assumed conditions - 10 or 25℃
- Initially used to predict steady-state BAFs; simplicity permits simulations to be generated as a continuous function of chemical log K_{ow}
- Can also be used dynamically to predict the kinetics of accumulation; of interest for simulating many experimental exposure protocols



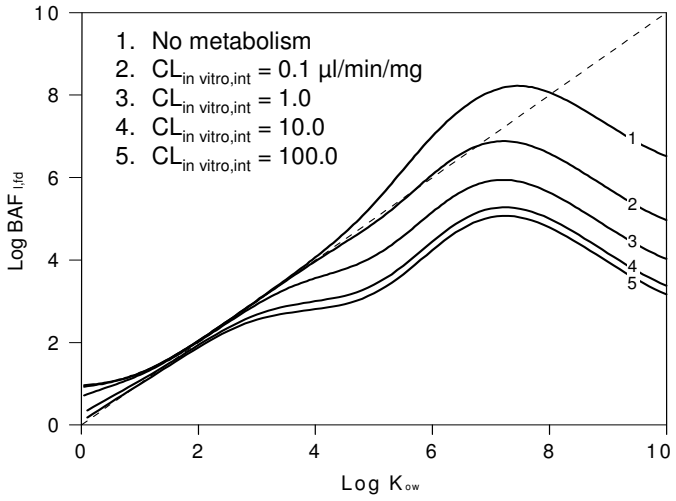
PBTK model parameters

- Starting sizes, gill physiology (ventilation volume), growth rates, and feeding rates were identical to those used by Arnot and Gobas (2004)
- Compartment sizes, partitioning values, and dietary uptake constants were adjusted to result in steady-state BAFs which, in the absence of metabolism, were nearly identical to those predicted by the one-compartment model

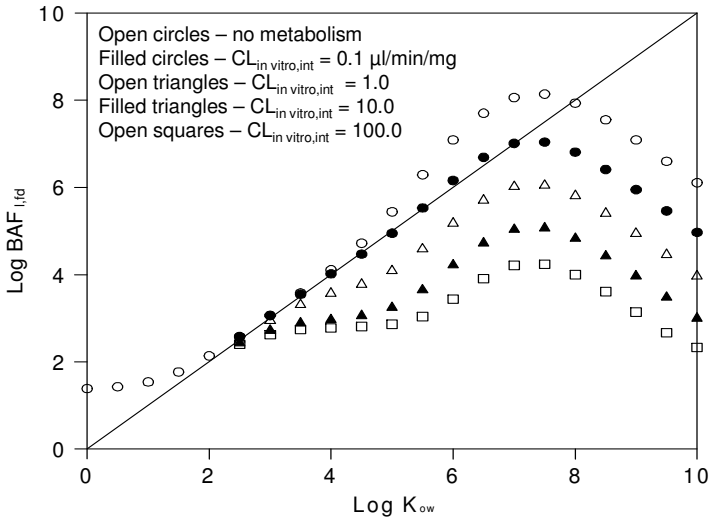
Bioaccumulation predicted by a one-compartment model in the absence of metabolism



Bioaccumulation predicted by the one-compartment model using hypothetical K_m and V_{max} values



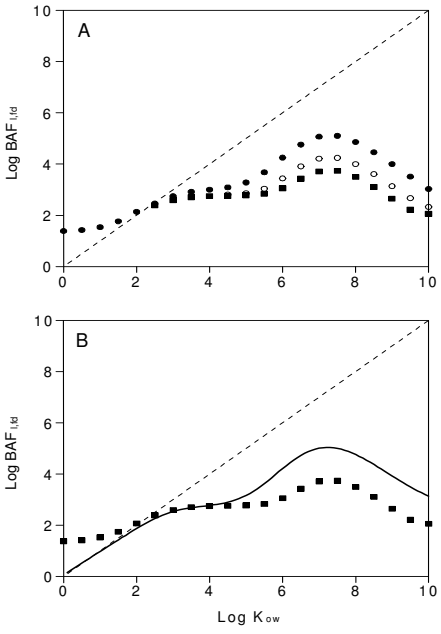
Bioaccumulation predicted by the PBTK model using hypothetical K_m and V_{max} values



Maximum effect of hepatic metabolism predicted by each model

A. Effect of blood flow limitation in the PBTK model
B. Maximum effect predicted by each model (due to blood flow limitations)

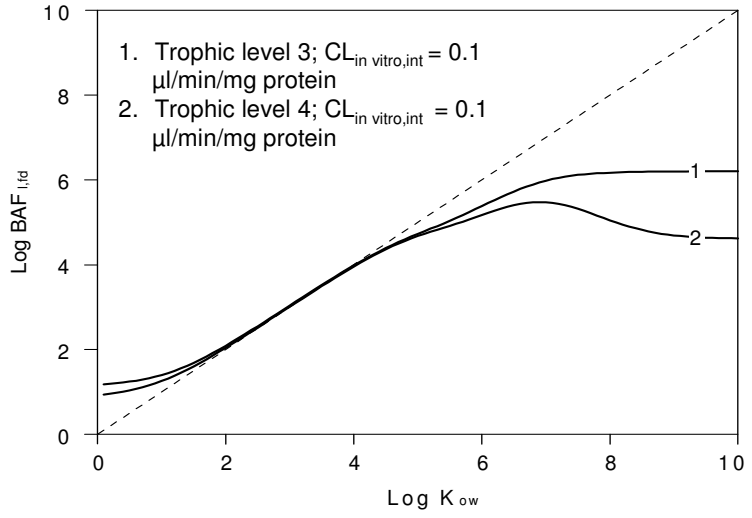
Filled circles – $CL_{in\ vitro,int}$ = 10.0 µl/min/mg
Open circles – $CL_{in\ vitro,int}$ = 100.0
Filled squares – $CL_{in\ vitro,int}$ = 10000.0
Solid line – $CL_{in\ vitro,int}$ = 10000.0



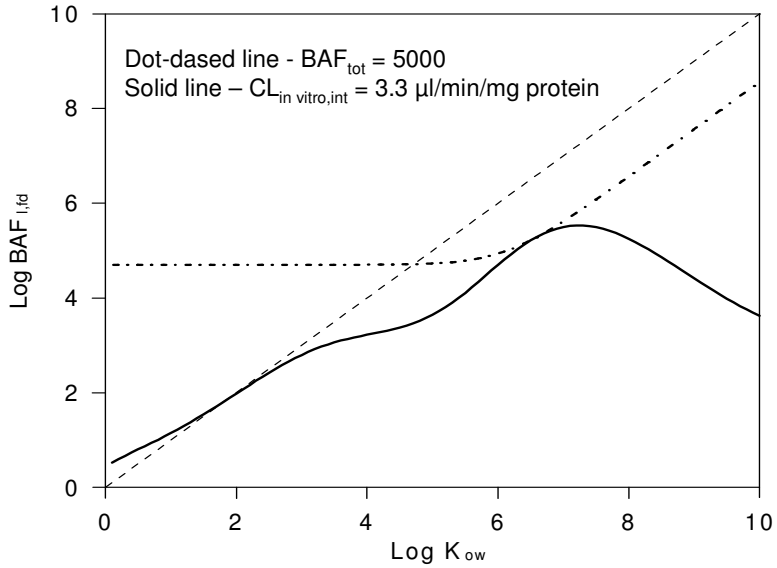
Summary and conclusions

- An established approach for performing *in vitro-in vivo* metabolism extrapolations was used to incorporate hypothetical *in vitro* data into two (one-compartment and PBTK) kinetic models for fish. The models were then used to simulate bioaccumulation across a range of log K_{ow} values.
- For a fixed level of *in vitro* activity, impacts on bioaccumulation predicted by the PBTK model are similar to those predicted by the one-compartment model at all but very high levels of activity.
- Direct incorporation of *in vitro* data into the one-compartment model is probably appropriate for most applications.

Potential applications: Interpretation of field residues



Potential applications: Screening



Remaining concerns

- *In vitro-in vivo* extrapolation procedures may be invalid in some, and perhaps many cases
  - extrahepatic metabolism; esp. gut, gills
  - protein binding
- Questions exist concerning the “best” type of *in vitro* test system
  - microsomes vs. S9 vs. hepatocytes
- *In vivo* data required to evaluate model predictions are lacking. In particular, we need paired *in vitro – in vivo* metabolism estimates for the same chemical and species.

Venous Equilibrium Liver Model

$CL_h = Q_h f_u CL_{in\ vivo,int} / (Q_h + f_u CL_{in\ vivo,int}$

Where,

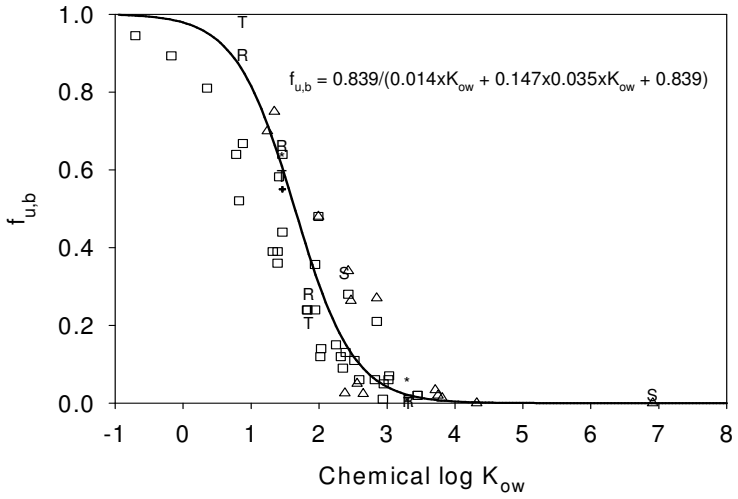
CL_h = hepatic clearance (ml blood cleared/time/g animal)

Q_h = liver blood flow (ml/time/g animal)

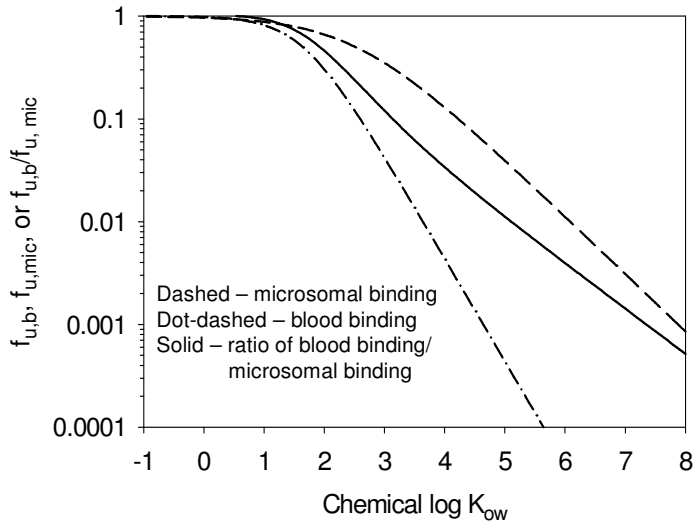
f_u = "free fraction", a term that corrects for effects of chemical binding (unitless)

$CL_{in\ vivo,int}$ = intrinsic activity of the enzyme, before taking blood flow limitations into account (ml liver cleared/time/g animal)

Chemical Binding in Plasma from Fish and Mammals



Net Result of Binding in Blood and in the In Vitro System Used to Assess Metabolism



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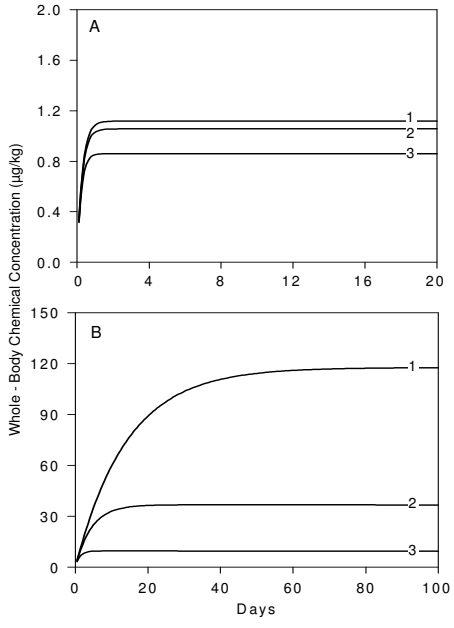
Current Activities and Next Steps

- SETAC Bioaccumulation Advisory Group; 11/04 to present
- ILSI/HESI Bioaccumulation Subcommittee; 4/05 to present
  - Planning meeting, Cincinnati, OH, 4/05
  - Workshop on BAF databases, Baltimore, MD, 11/05
  - Workshop on the use of in vitro data in BAF prediction, San Diego, CA, 3/06
- SETAC Europe meeting, The Hague, The Netherlands, 5/06
- ECVAM solicitation of interest for funding

Effect of metabolism on kinetics predicted by the one-compartment model

A. Log K_{ow} = 2.0
B. Log K_{ow} = 4.0
1. No metabolism
2. $CL_{in\ vitro,int}$ = 1.0 µl/min/mg
3. $CL_{in\ vitro,int}$ = 10.0



Potential applications: Screening