In vitro to in vivo extrapolation of hepatic metabolism in fish: an inter-laboratory comparison of in vitro methods

ILSI Health and Environmental Sciences Institute

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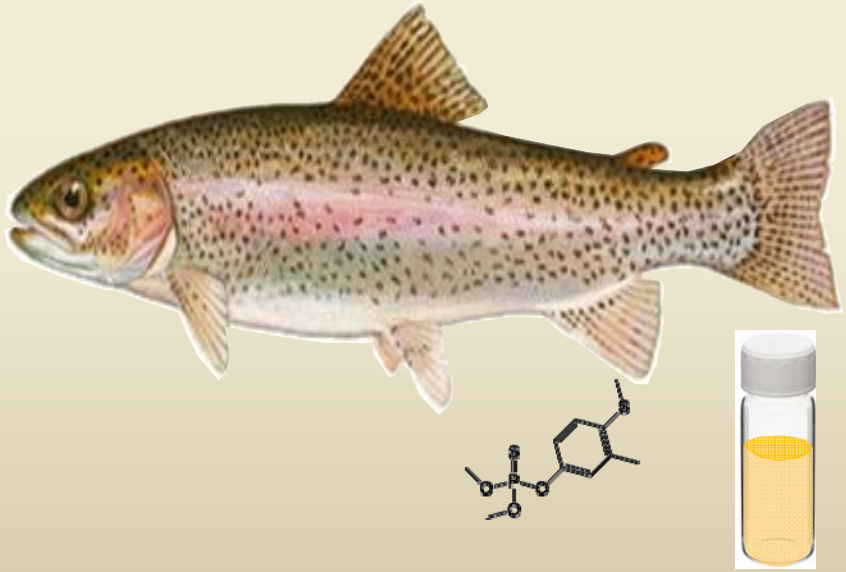
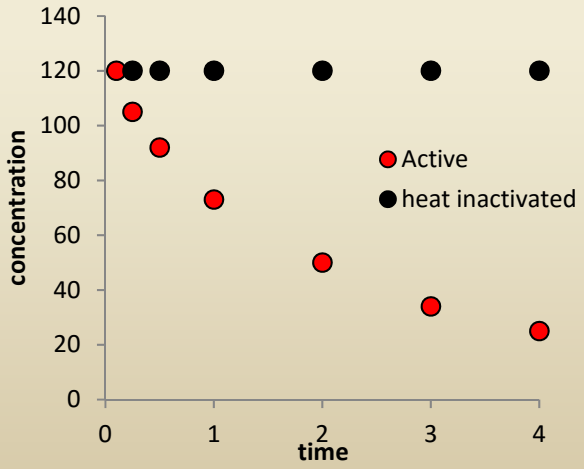




What is the need?

- Chemicals are assessed for their potential to bioaccumulate

  Accumulation of a chemical in an organism is the result of absorption, distribution, metabolism, and excretion (ADME)

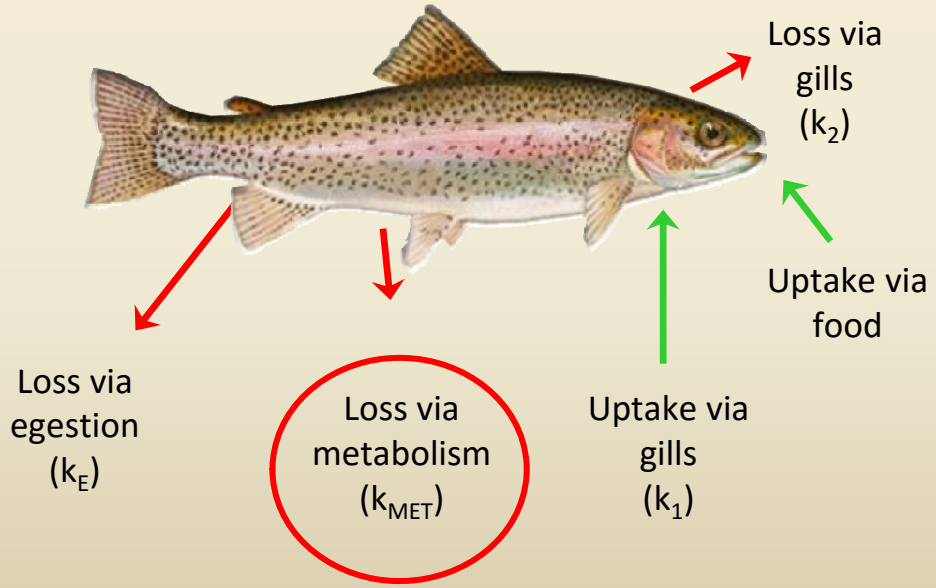
- Bioconcentration factors (BCF) are used as a surrogate for bioaccumulation

  Only waterborne uptake

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

- BCF predictions based on log K_{ow} are often good EXCEPT when the chemical is metabolized

  Biotransformation is the single largest uncertainty in BCF predictions!



In Vitro clearance assays can be used to estimate the rate of chemical biotransformation – but there is a lack of reliable and reproducible data!

HESI: A Platform to Meet Protocol Standardization Needs

REACH endpoint specific guidance – Bioaccumulation (ECHA, 2012)

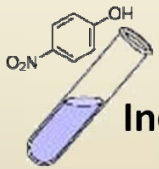
"In vitro methods have the potential to provide important data on bioaccumulation assessments…These methods may become an important part of future test strategies, but their applicability is currently limited due to the lack of standardised protocols, limited validation based on small data sets. Further evaluation work is necessary before they can be recommended for use within an ITS."

In vitro method: Application for Bioaccumulation Assessments in fish

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

- Hepatocellularity (cells/g liver)
- S9 protein content (protein/g liver)

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

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

Calculate hepatic clearance (L/h/kg fish)

- Apparent volume of distribution (L/kg)

Calculate 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

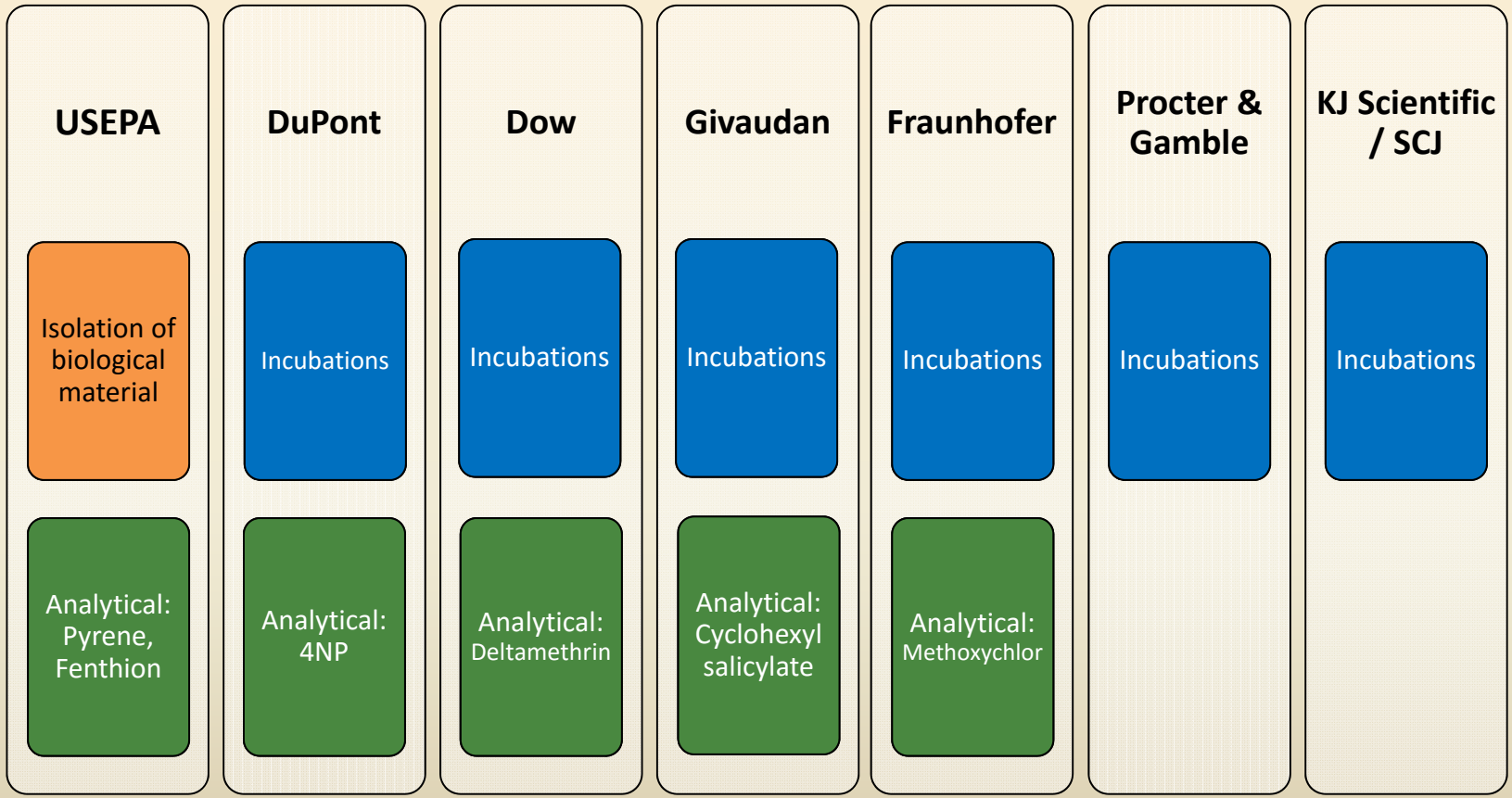
Fay et al, 2014; Nichols et al., 2013; Johanning et al, 2012; Cowan-Ellsberry et al., 2008; Han et al., 2007

Overall aims of the project

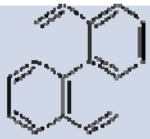
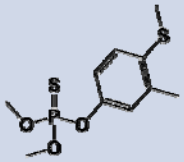
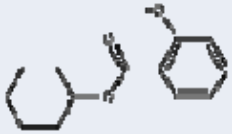
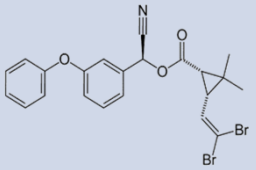
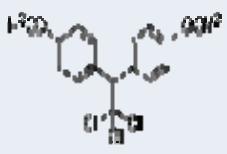
- Compare the performance of rainbow trout S9 and cryopreserved hepatocytes within and across laboratories
  - Reliability of substrate depletion assays
  - Evaluate if S9 and hepatocytes give similar answers

- Develop OECD Test Guidelines (OECD Project 3.13; EC and US lead)
  - Standardized protocols

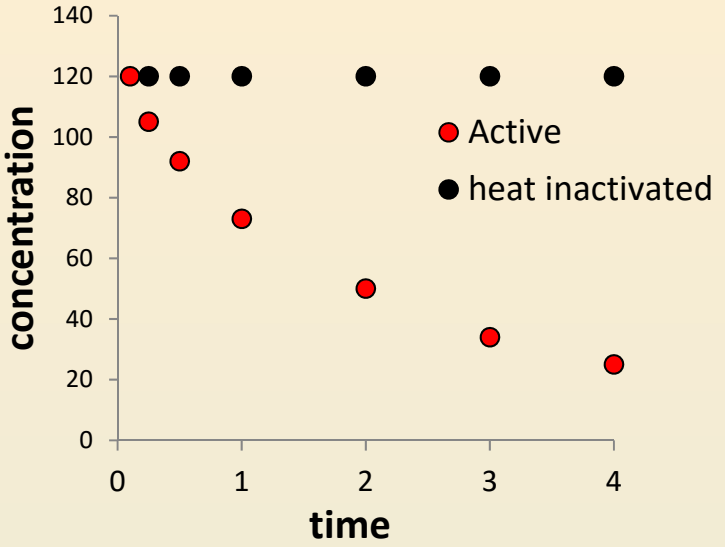
Ring Trial: Participating laboratories

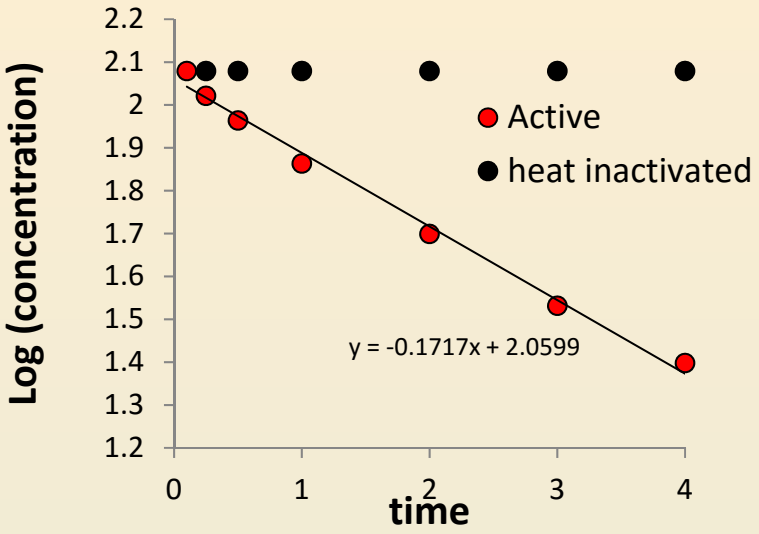


Chemical Selection

Chemical	Structure	Log K_{ow}	Empirical BCF
Pyrene		4.9	86-1612
4-n-nonylphenol		5.8	297
Fenthion		4.1	26-717
Cyclohexyl salicylate		4.7	320-480
Deltamethrin		6.2	147
Methoxychlor		5.1	174

Calculation of in vitro intrinsic clearance

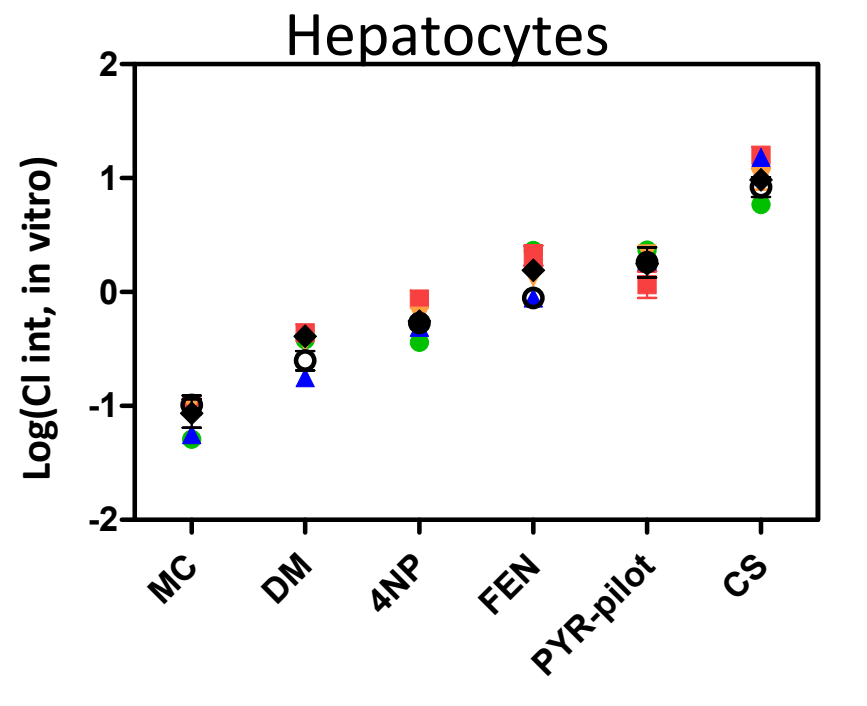




- Determine the concentrations (μM) at each time point
- Plot $\log_{10}$ (concentration) against time
- -2.30 x slope = elimination rate constant = k_e
- **elimination rate/ biological concentration = in vitro intrinsic clearance**

  k_e/ concentration biological material = *Cl_{int} (ml/h/10^6 cells)*
  or Cl_{int} (ml/h/mg protein)

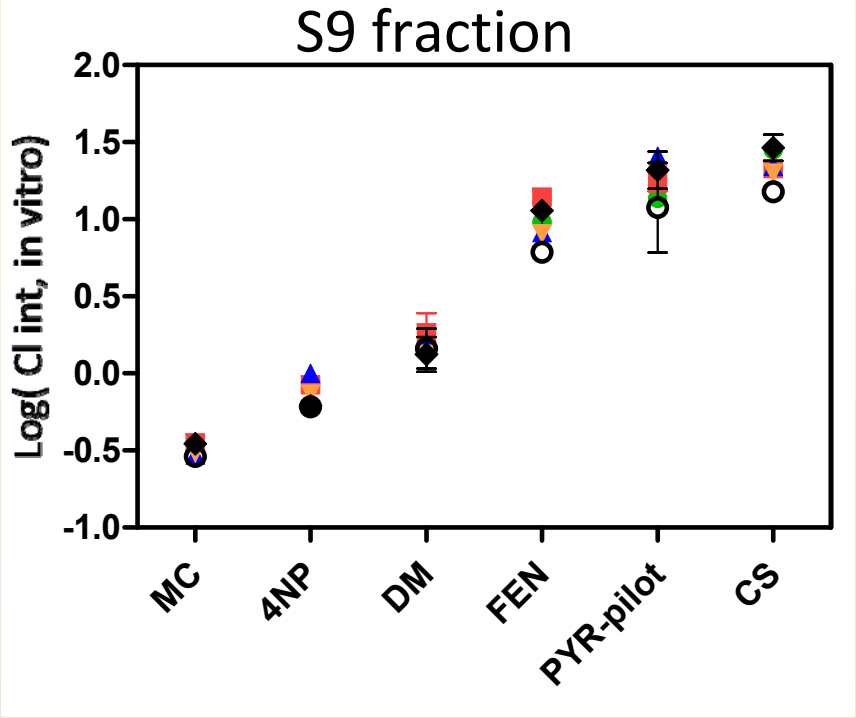
In vitro Intrinsic clearance results - Hepatocytes



- 6 labs
- 6 chemicals
- Triplicate assays for each chemical/ lab

- Chemicals had a range of clearance rates (0.09 – 10.7 mL/h/10^6 cells)
- Labs determined similar rates for each chemical
- Rates for each chemical had similar variability

In vitro Intrinsic clearance results – S9



- Chemicals had a range of clearance rates (0.3 – 28.7 mL/h/mg S9)
- Similar order of increasing rates as hepatocytes
- Labs determined similar rates for each chemical
- Rates for each chemical had similar variability

Preliminary assay reliability results (% CV)

	PYR-pilot	4NP	CS	FEN	DM	MC
HEP Intra-lab	(4 – 53) Avg 23%	(6-24) Avg 17%	(4-37) Avg 23%	(5-35) Avg 15%	(5-34) Avg 17%	(6-50) Avg 21%
HEP Inter-lab	22%	26%	37%	40%	30%	29%
S9 Intra-lab	(3-48) n=5 Avg 19%	(8-21) Avg 14%	(8-34) Avg 18%	(0.4 -21) Avg 9%	(4-52) Avg 29%	(0.4-11) Avg 5%
S9 Inter-lab	23%	18%	21%	29%	13%	10%

Intra-lab variability (average) 5-29%

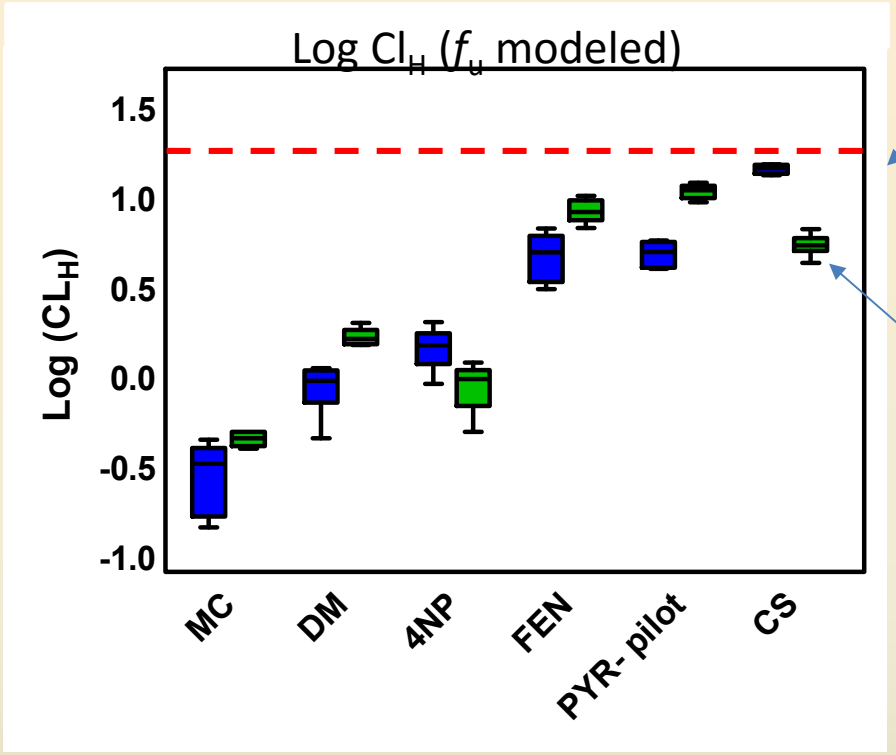
Previous hepatocyte ring trial Intra-lab variability 4-30 % CV

Inter-lab variability 10-40 %

Previous hepatocyte ring trial Inter-lab variability 27-61 % CV

HEP assays may have somewhat greater inter-lab variability, as expected (cell counting vs dilution of S9); however, both systems demonstrate good agreement

Hepatic Clearance results- f_u modeled



Blood flow limitation
= 18.2 L/d/kg
(log 18.2 = 1.26)

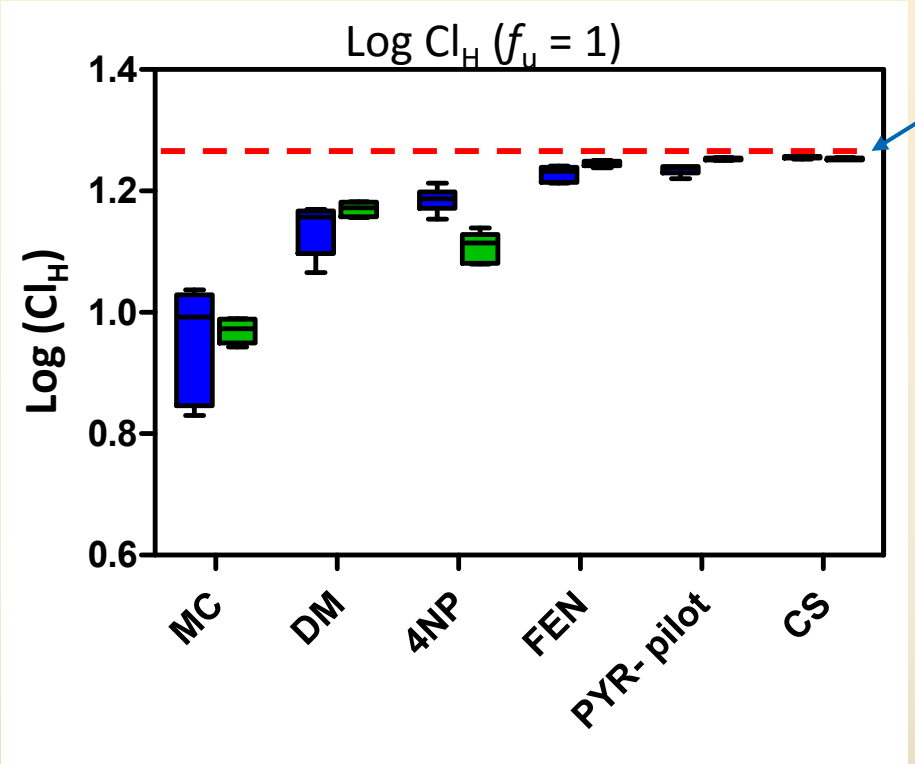
HEP

S9

Rapid depletion of CS required reducing S9 protein concentration (0.25 mg/ml); may be saturating

- Most rapidly cleared compounds are approaching the blood flow limitation
- Hep and S9 predictions are in close agreement(<2x); most different is CS

Hepatic Clearance results- f_u =1



Blood flow limitation = 18.2 L/d/kg (log 18.2 = 1.26)

HEP

S9

- Excellent agreement (<1.2 fold) between Hep and S9 predictions
- More chemicals approach blood flow limitation

BCF results- comparison to empirical values

	Log K_{ow}	Predicted BCF (k_{met}= 0)	Empirical BCF (L/kg)	Avg HEP BCF (L/kg)		Avg S9 BCF (L/kg)	
				f_u modeled	f_u = 1	f_u modeled	f_u = 1
Fenthion	4.1	621	26-717*	283	118	197	114
Cyclohexyl salicylate	4.7	2370	320-480	217	181	463	182
Pyrene	4.9	3640	86-1612*	676	214	322	206
Methoxychlor	5.1	5441	174	3964	452	3445	426
4-Nonyl phenol	5.8	16549	297	2909	321	3905	382
Deltamethrin	6.2	22900	147	4186	315	2472	292

* Several BCF studies with different species

- f_u modeled - large reductions from predicted BCFs, may still be higher than empirical BCFs
- f_u = 1 - very close agreement with empirical BCFs, especially when clearance rates approached blood flow limitation
- Even slowly metabolized compounds (e.g., methoxychlor) predictions are greatly improved

Conclusions

- Primary goal was to determine if in vitro substrate depletion method was a reliable method (different people in different labs get the same answer)

YES!

For both S9 and HEP, Inter-laboratory variability was 10 - 40% CV ; **avg 25 % CV**

- BCF predictions using the S9 and HEP in vitro assays were within ~2x agreement for the modeled binding, even better (<1.2x) for the f_u=1 assumption.
- Most rapidly cleared compounds were in best agreement with empirically measured BCFs
- BCF predictions for slowly cleared compounds still greatly improved by incorporating in vitro intrinsic clearance
- Future steps
  - Publication of 2 manuscripts (Results and statistical study design)
  - Development of OECD test guidelines for in vitro assays (Hep & S9)
    Development of a guidance document for the test guidelines

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