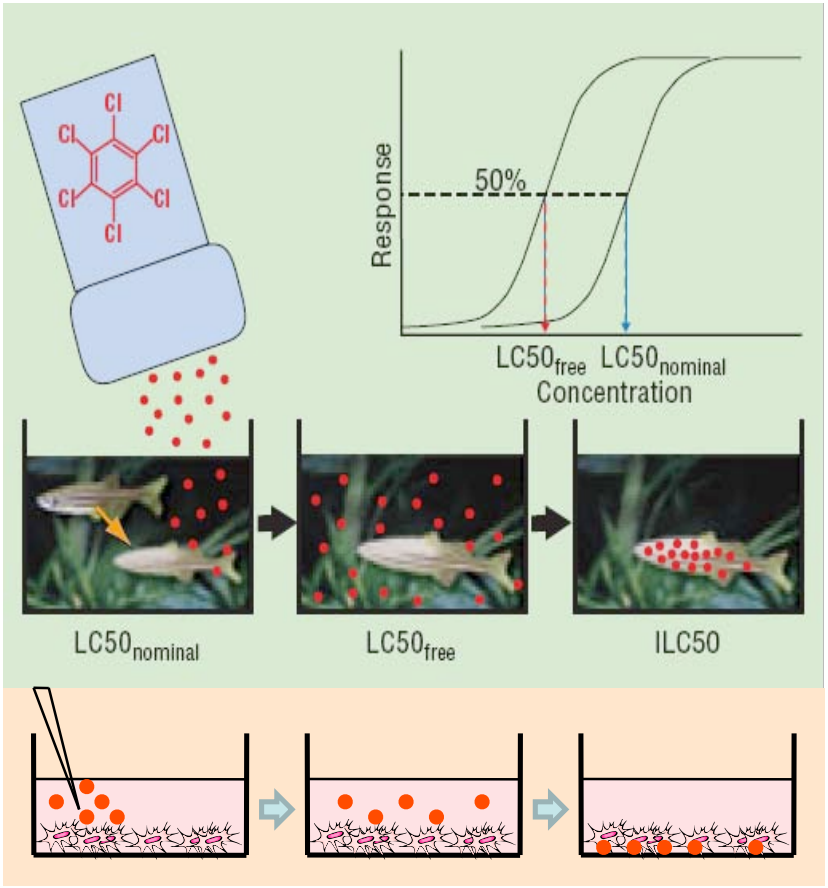
Universiteit Utrecht

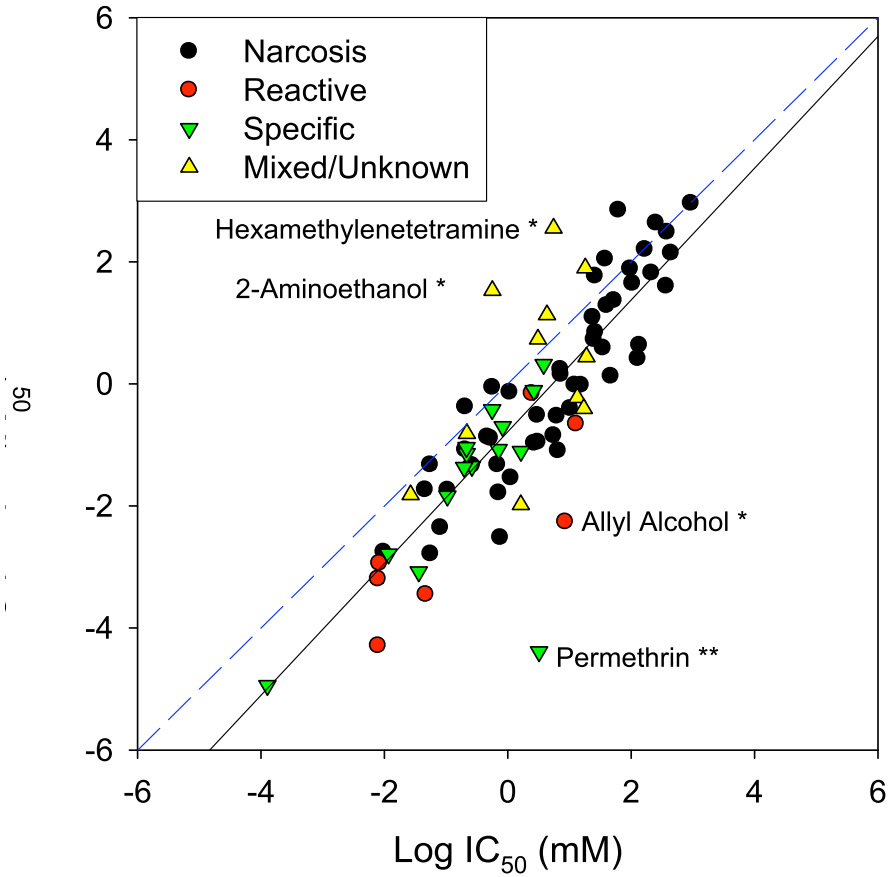
Free Concentrations in *In Vitro* Cytotoxicity Assays

***Nynke Kramer*, Bas Blaauboer and Joop Hermens**

Institute for Risk Assessment Sciences / Division Toxicology

Free Concentrations

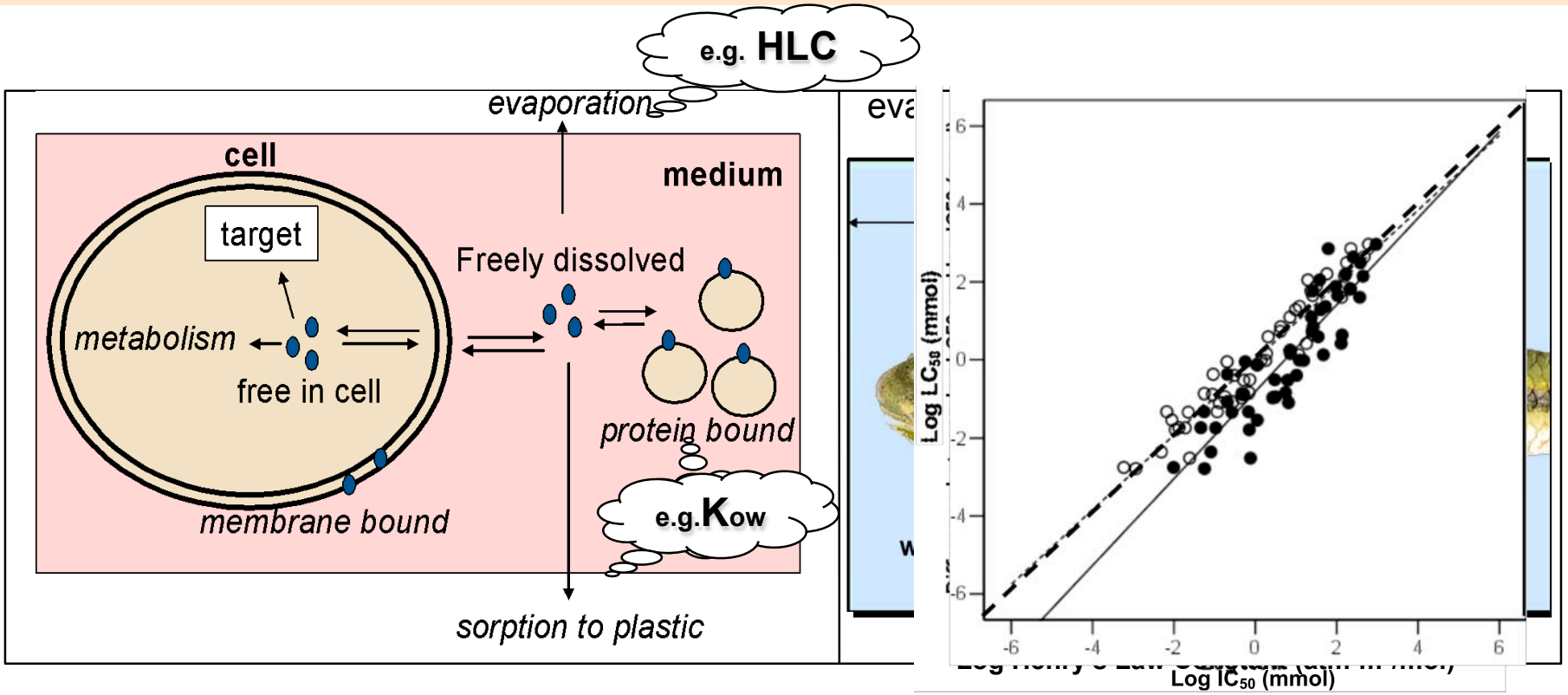




Escher and Hermens (2004) EST 38 455-462A.

Kramer et al. (2008) Submitted.

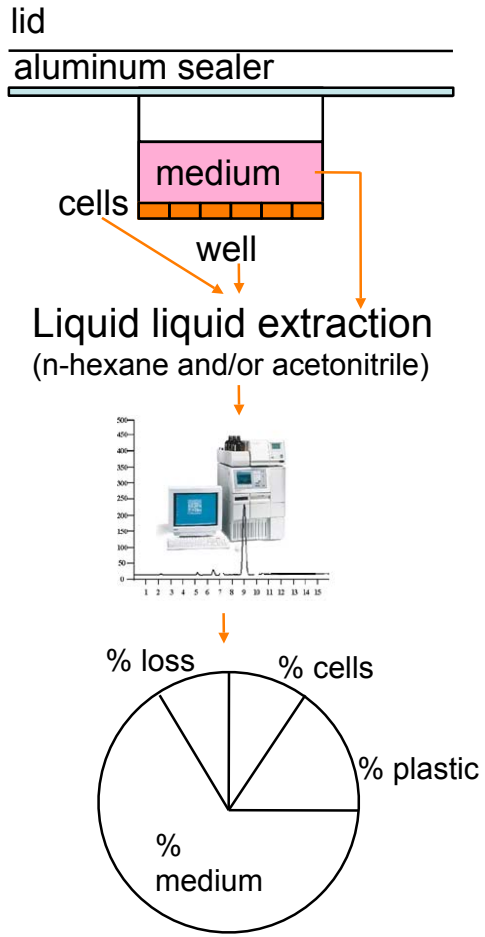
Differences *In Vitro/In Vivo*



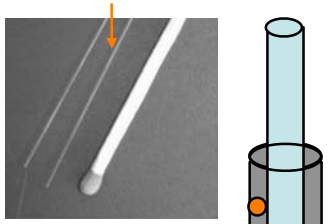
- **Aim:** Assess what **system components** and what **physicochemical properties** determine the free concentration and cytotoxicity of PAHs to Balb/c 3T3 cells in a NRU assay.

Measuring and modeling exposure

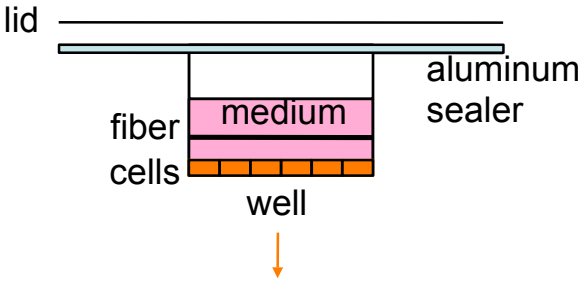
Partitioning to Components:



Measuring Free Conc. *In Vitro*:
Solid Phase Microextraction

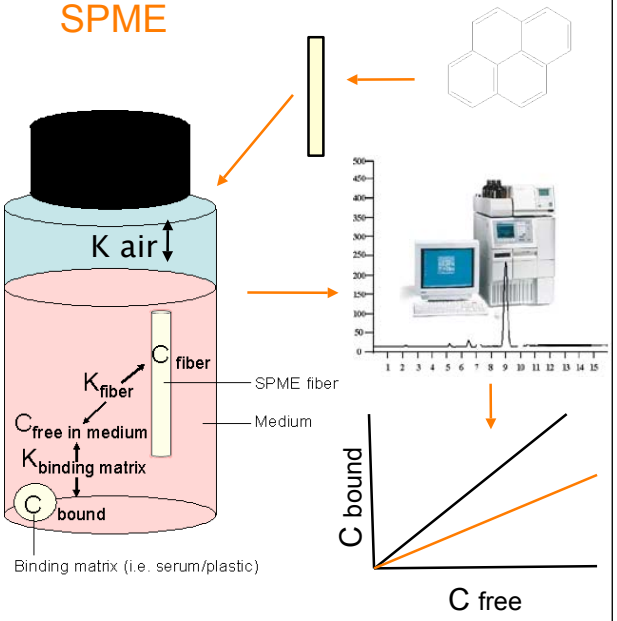


$C_{fiber}/C_{free\ (aq)} = K_{fiber}$



NRU to test for cytotoxicity

Modeling Free Conc. *In Vitro*
SPME

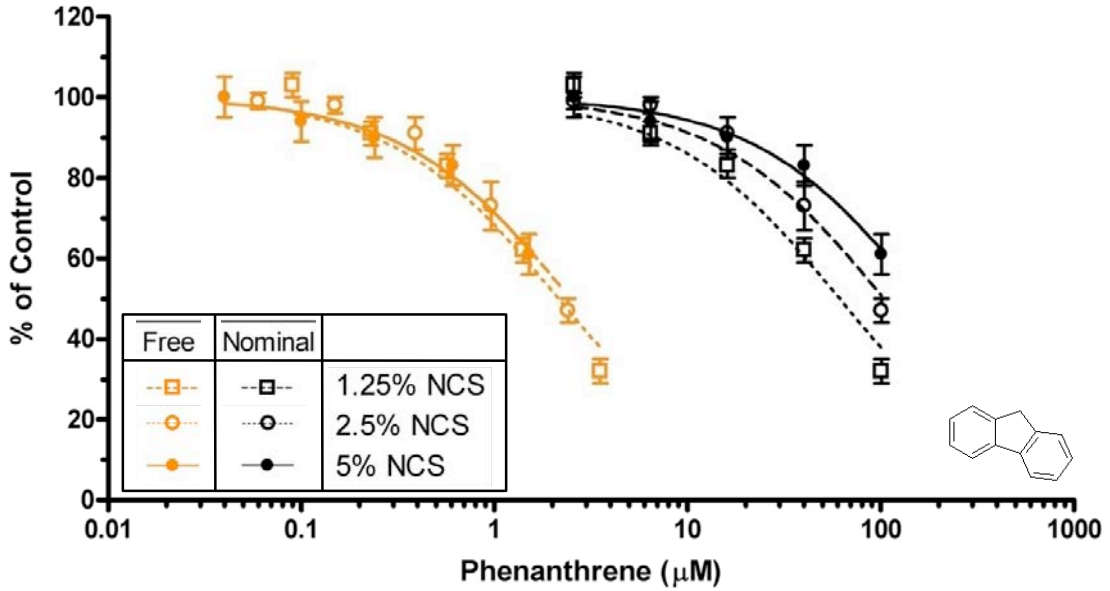


Partition coefficient:
S phase 1 ⇄ S phase 2

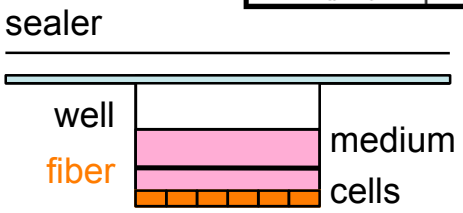
$$K = \frac{[\text{S phase 1}]}{[\text{S phase 2}]}$$

Model basis:
$F = 1/(1 + Ka \cdot Ca\ldots)$

Effects of changes in C_{free}

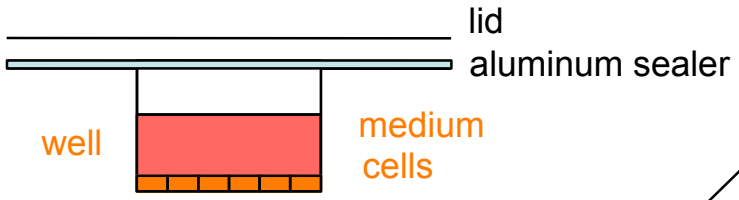


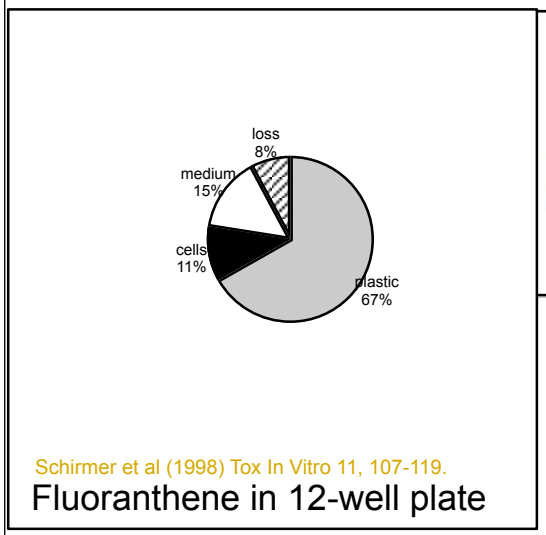
NCS (%)	1.25		2.5		5	
Log EC_{50}	1.79 ± 0.06	0.33	2.02 ± 0.04	0.40	2.22 ± 0.03	0.40
EC_{50} (μM)	61.3	2.15	104.1	2.49	165.5	2.48

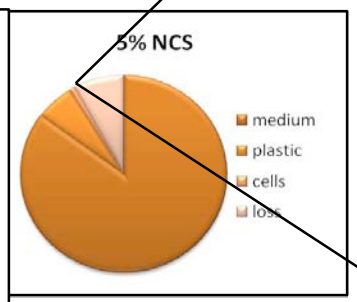


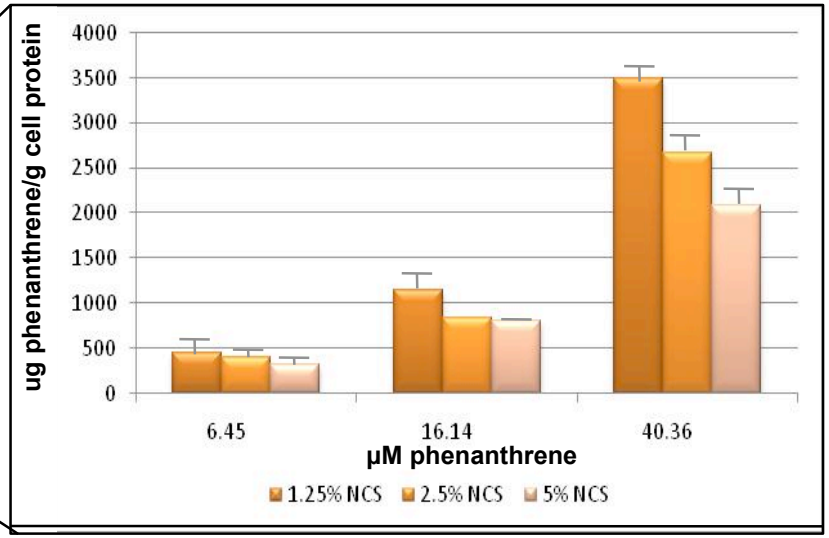
	NCS Level (%)		
	1.25	2.5	5
% free, measured	**3.50 ±0.75%**	**2.39 ±1.01%**	**1.02 ±0.20%**

Partitioning *in vitro*









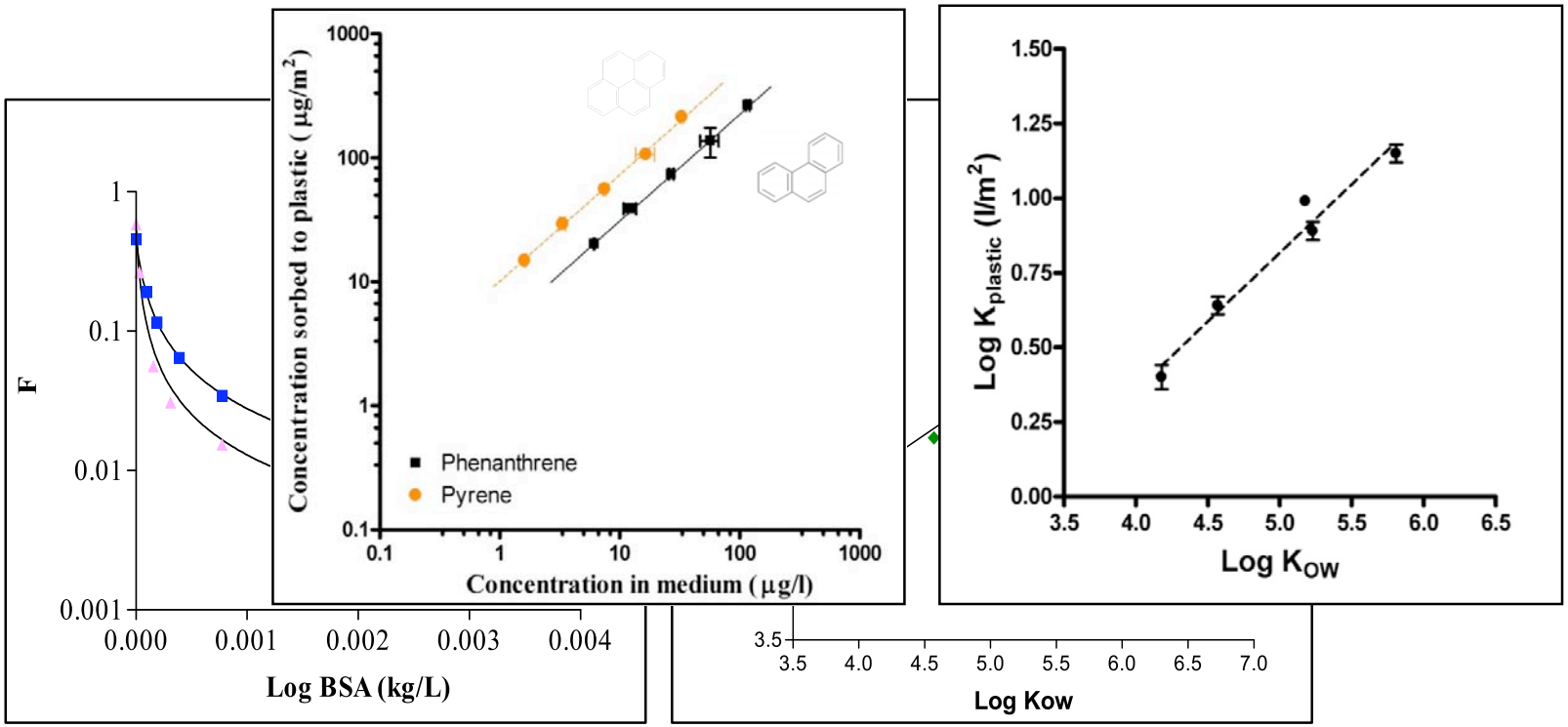
Schirmer et al (1998) Tox In Vitro 11, 107-119.

Fluoranthene in 12-well plate

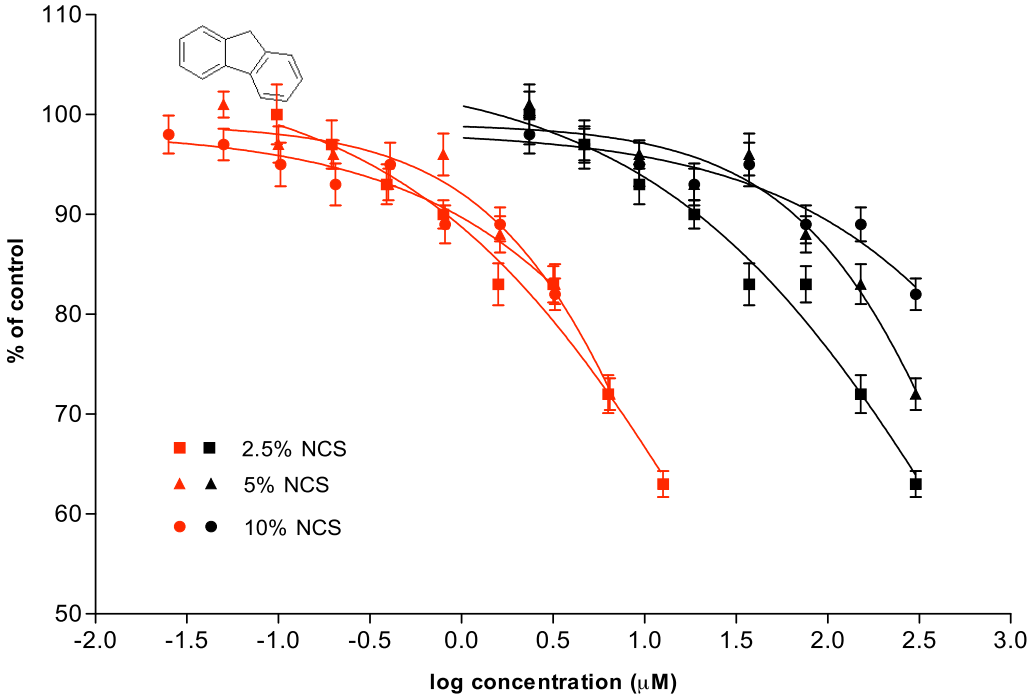
Partitioning Coefficients

Serum Binding

Plastic Binding



Partitioning *in vitro*



Kow

log K_f	log K_a ± SE (l/kg)
3.91	4.52 ± 0.02

$$\text{Free fraction} = \frac{1}{1 + (Ka * Ca)}$$

	2.5	5	10
EC_{20} (μM)	57.54	199.53	389.05
EC_{20} (μM) free	2.41	4.27	4.20

NCS Level (%)				
2.5		5		10
model	meas. 2.39 %	model	meas. 1.02 %	model 0.96%

Conclusions

- When using *in vitro* tests to quantitatively predict acute *in vivo* toxicity, it is suggested to a.o.:

- Account for free concentrations *in vitro*

  - Standardization / Good Cell Culture Practice (GCCP)
  - Measure / Model free concentrations accounting for:
    * Serum protein binding
    * Well plate plastic binding
    * Cell concentration
    * Headspace
  - SPME may be used for both measurement of free concentrations directly and partition coefficients
  - K_{ow} and HLC can be used as indicators for alerts / correctors of free concentrations.

Thanks for your attention

Thanks also to: Bas Blaauboer and Joop Hermens