

Bioavailability assessment of organic pollutants in biaccumulation and toxicity studies using solid phase extractions

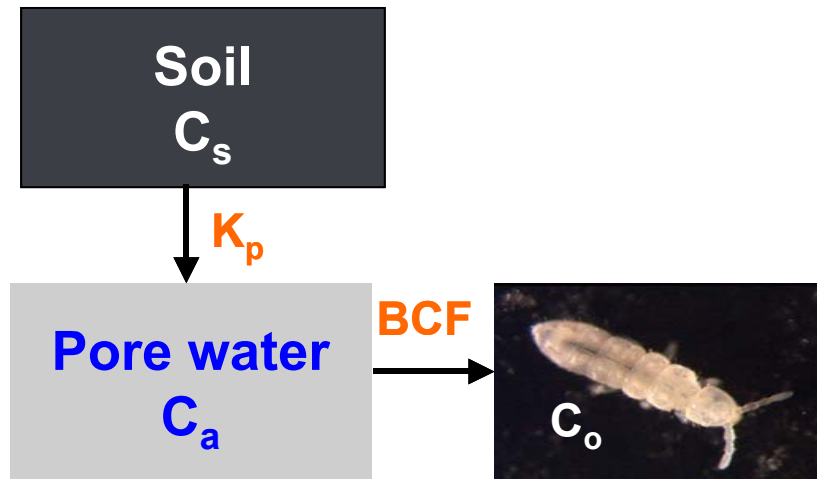
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With contributions from

PhD students: Thomas ter Laak, Leon van der Wal, Heather Leslie,
Minne Heringa (Philipp Mayer and Wouter Vaes)
Technicians: Arjan Barendregt, Theo Sinnige and Frans Busser

Site specific risk assessment

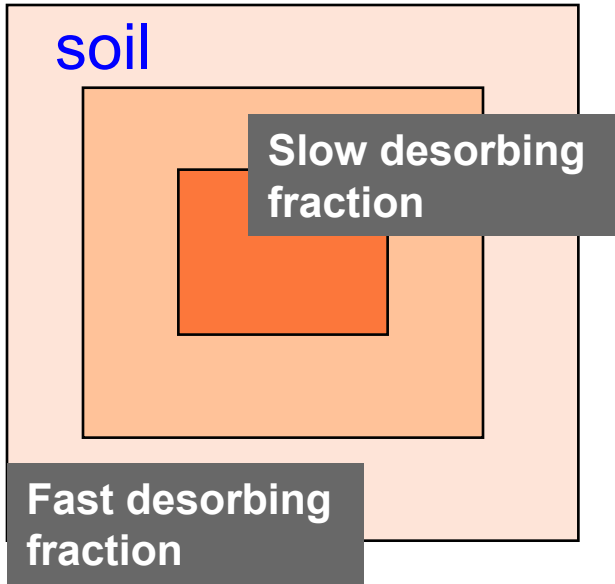


Bioavailability issue:

Partition coefficient soil or sediment (K_p) – water can be much higher than predicted from K_{ow} , because of:

- Aging effects
- Presence of strongly binding components in sediment (soot)

Methods to measure “bioavailability”



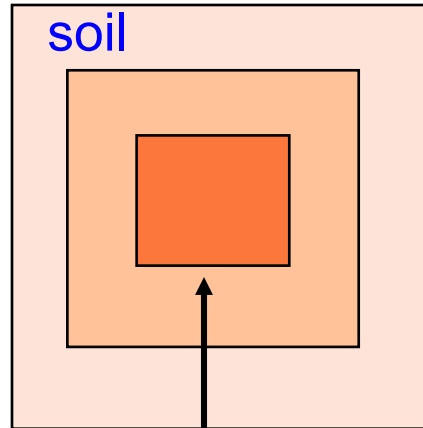
Depletive extractions

- Tenax (Pignatello, Cornelissen)
- Cyclodextrin (Reid)
- SFE (Hawthorne)

Concentration or amount in fast desorbing fraction



Methods to measure “bioavailability”

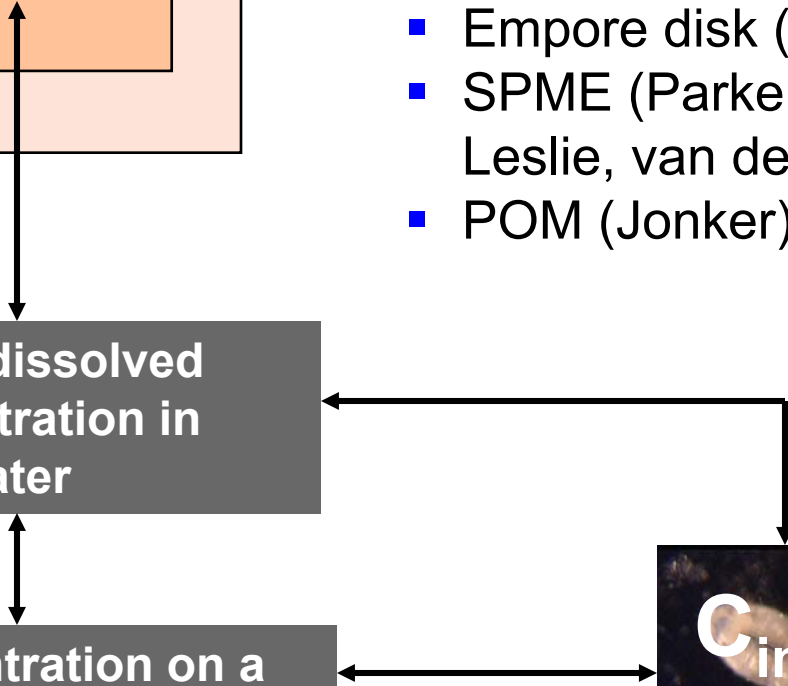


Non-depletive extraction (partition based / biomimetic)

- SPMD (Huckins, Sodergren)
- Empore disk (Verhaar, Van Loon)
- SPME (Parkerton, Lanno, Vaes, Mayer, Leslie, van der Wal, Heringa, ter Laak)
- POM (Jonker)

Freely dissolved concentration in pore water

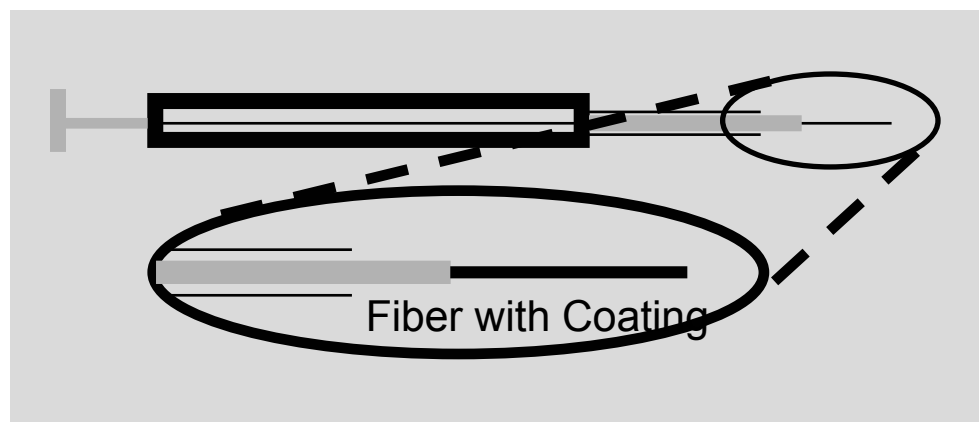
Concentration on a hydrophobic phase-surrogate organism



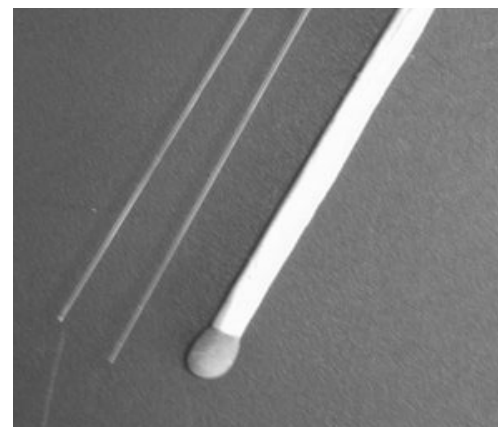
Sampling techniques: SPME

SPME: Solid phase micro-extraction:

- Polymer coated fibers (polymer PDMS, polyacrylate)
- Hydrophobic chemicals diffuse to coating
- Thermal desorption in injector of GC or solvent desorption for HPLC

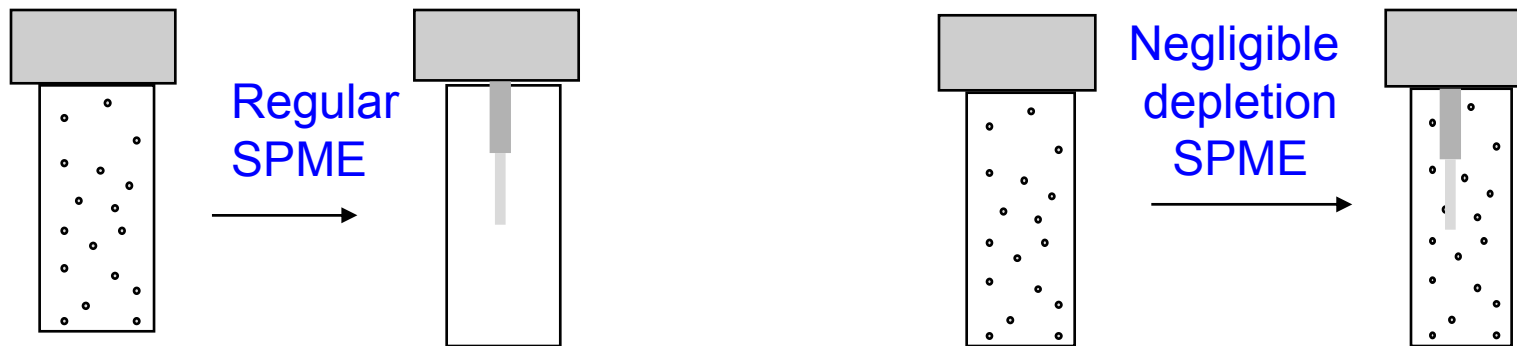


commercial fiber



disposable fiber

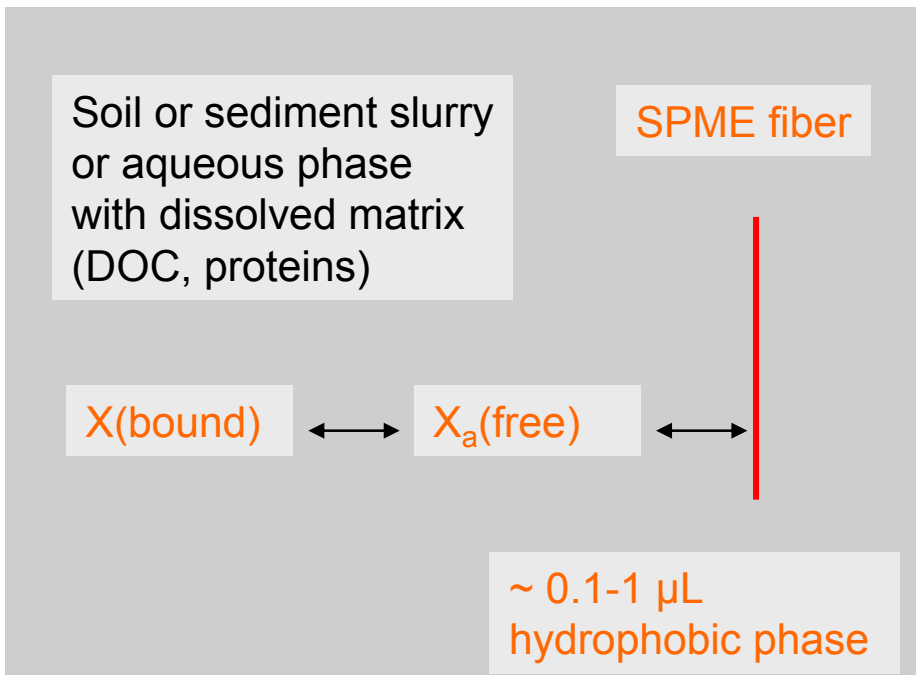
Negligible depletion (nd) SPME for measuring free concentration and estimating internal concentrations



negligible depletion by:

- small volume of fiber coating
- short exposure times

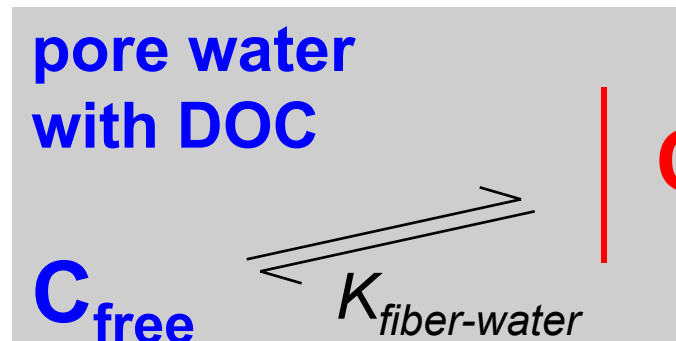
Negligible depletion (nd) SPME for measuring free concentration and estimating internal concentrations



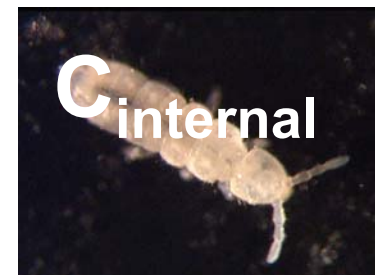
Negligible depletion SPME:

- Only free X_a partitions to fiber.
- If extraction is negligible (< 5 %): no shift in equilibrium bound-free

Negligible depletion (nd) SPME for measuring free concentration and estimating internal concentrations



C_{fiber}

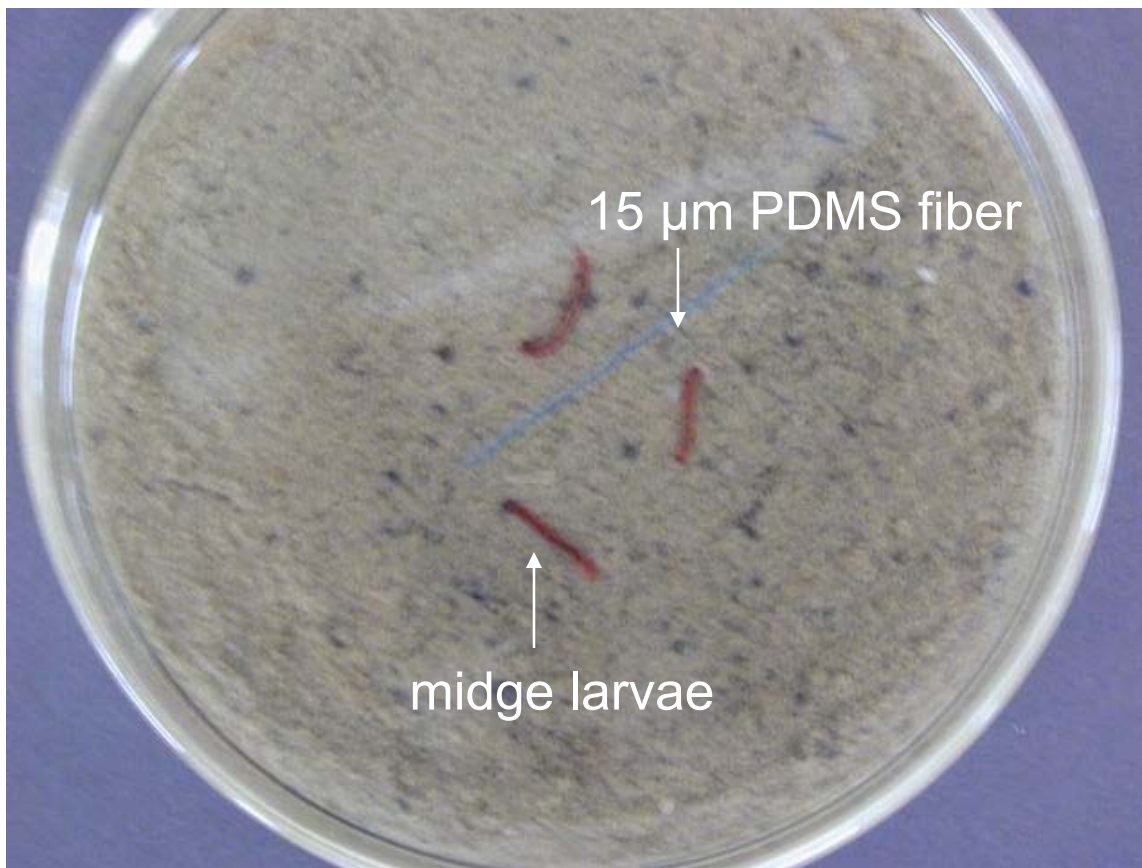


calibration

measuring free concentration

estimating internal concentrations

Application of nd-SPME to measure internal concentrations in sediment organisms (Heather Leslie)



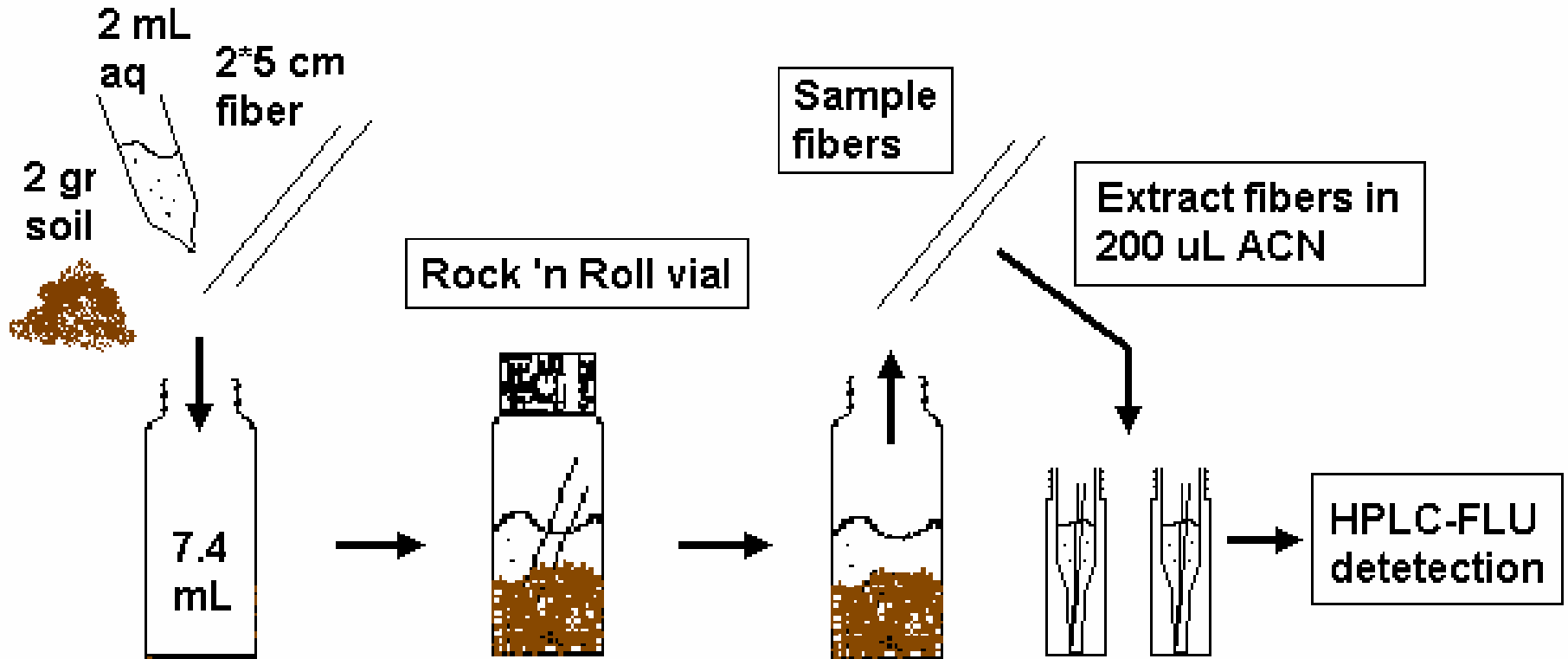
Leslie, H.A.; Oosthoek, A.J.P.; Busser, F.J.M.; Kraak, M.H.S.; Hermens, J.L.M. *Environ. Toxicol. Chem.* 2002, 21, 229-234.
Leslie, H.A.; Ter Laak, T.L.; Busser, F.J.M.; Kraak, M.H.F.; Hermens, J.L.M. *Environ. Sci. Technol.* 2002, 36, 5399–5404.

Application of solid phase micro-extraction to measure
freely dissolved concentration of PAHs in soil

PhD project: Thomas ter Laak (2005)

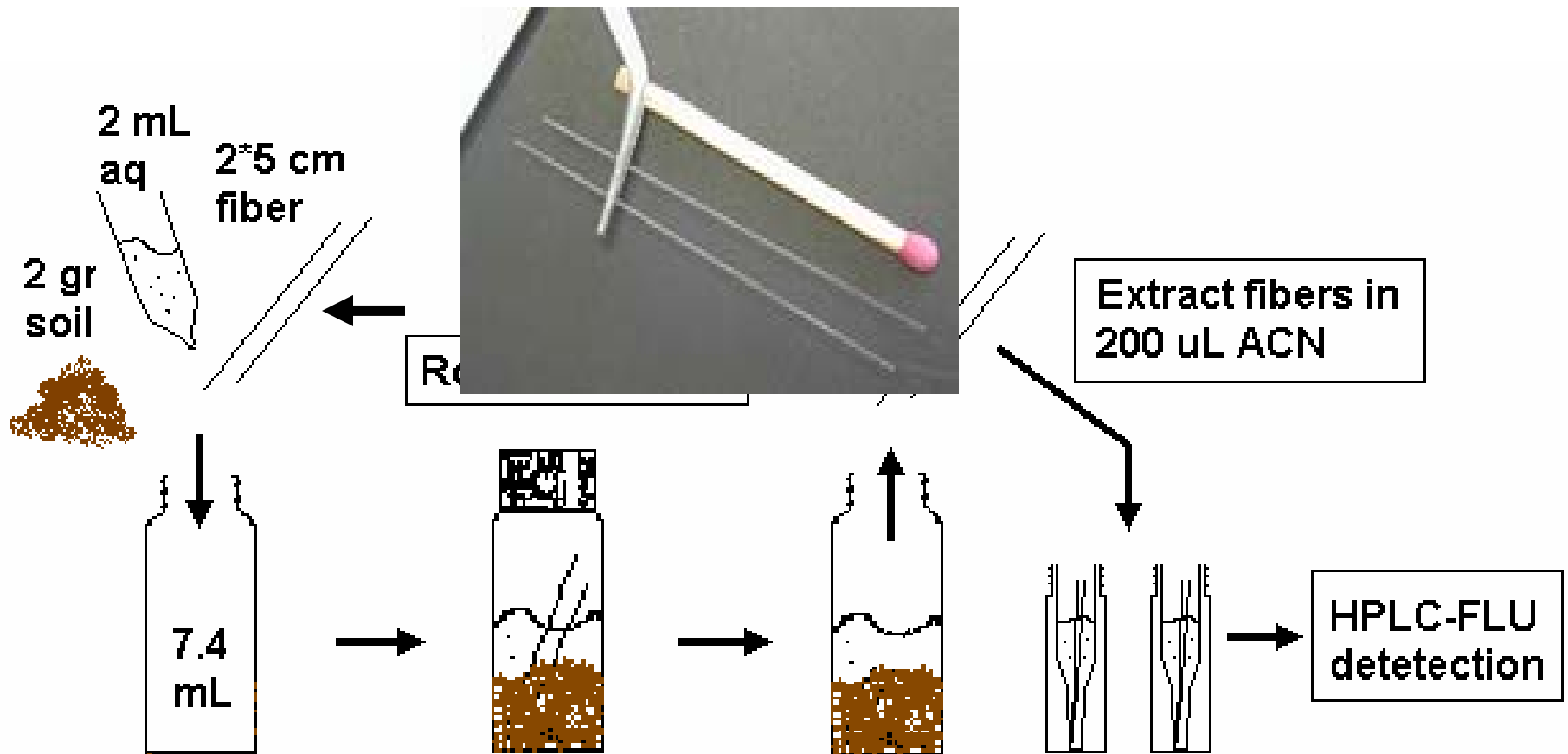
Technician: Arjan Barendregt

Assessing free concentrations of PAHs in soil

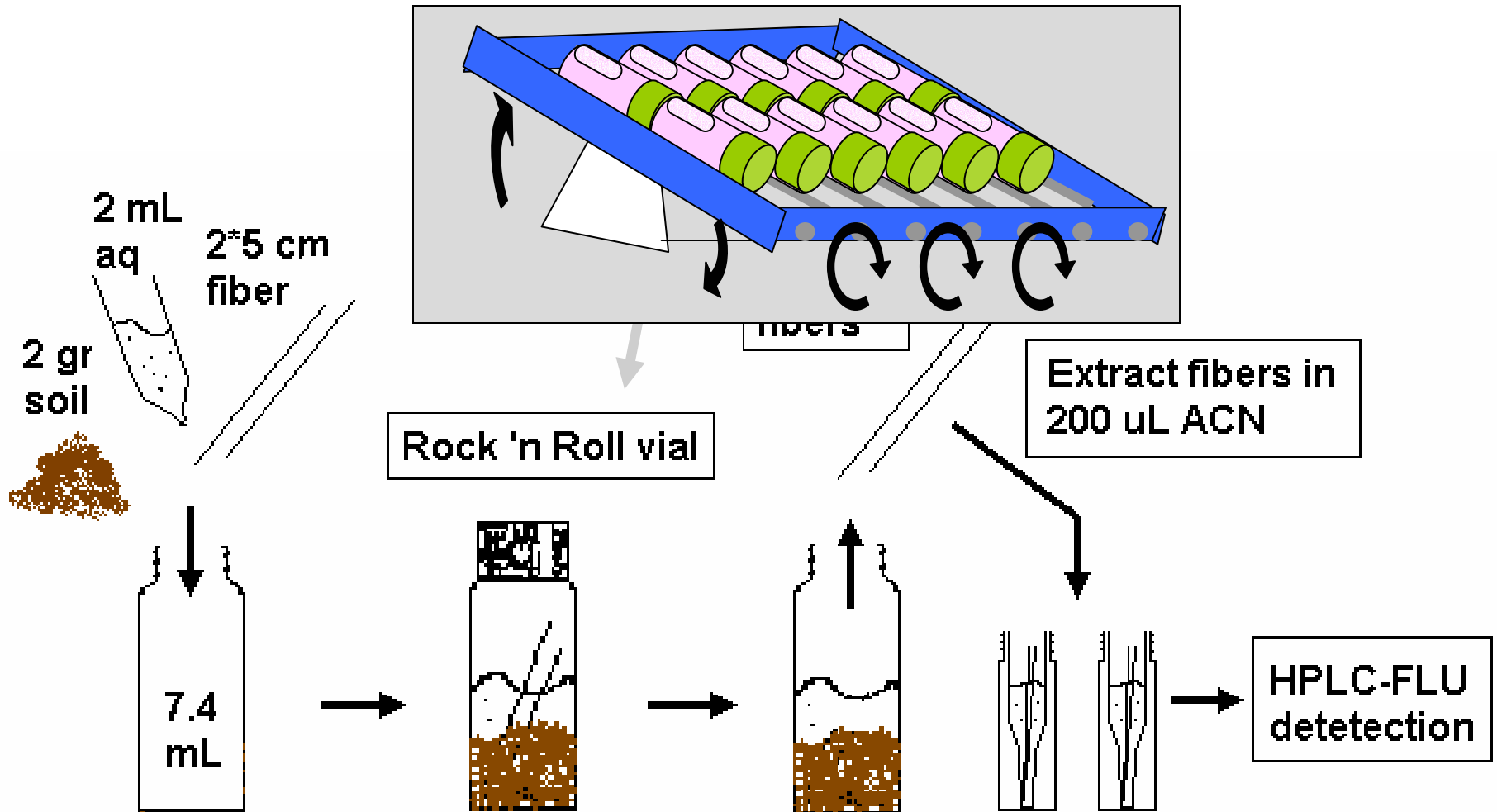


(based on: Mayer, 2000, van der Wal, 2004)

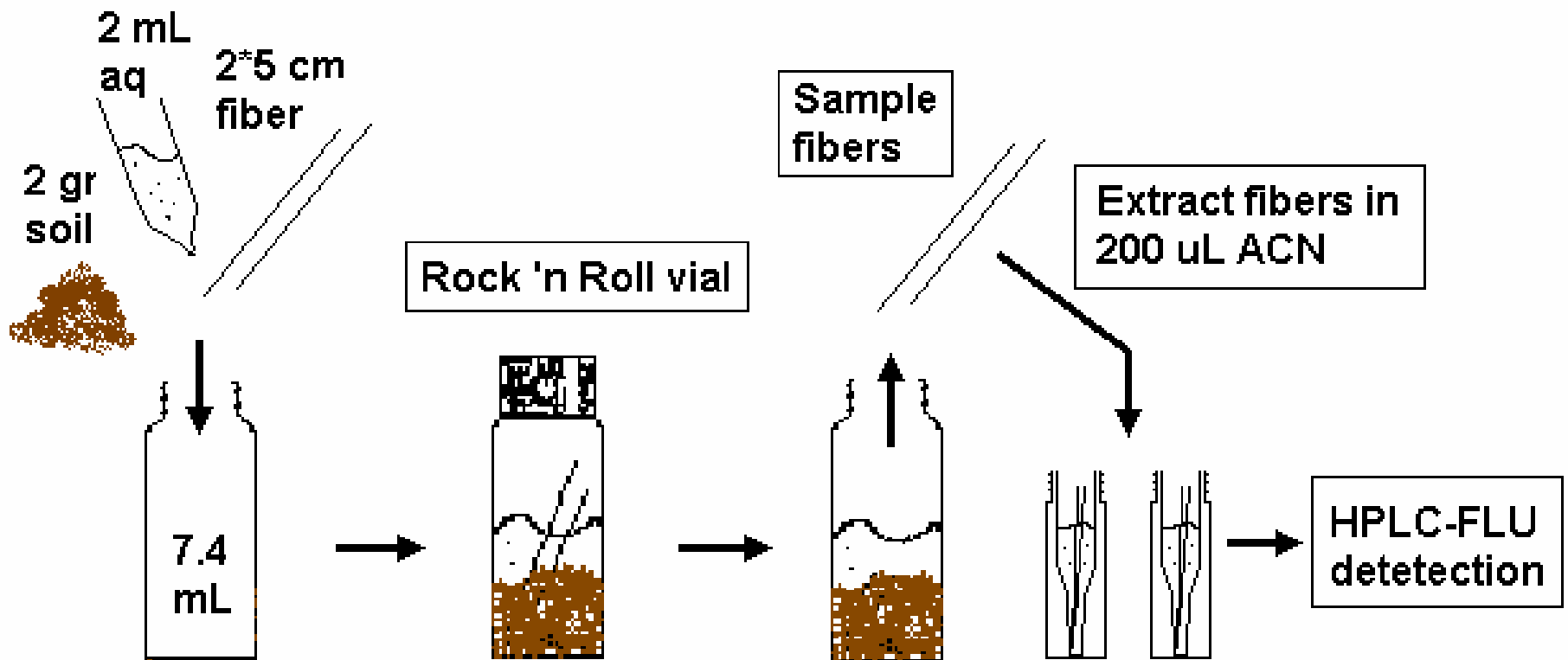
Assessing free concentrations of PAHs in soil



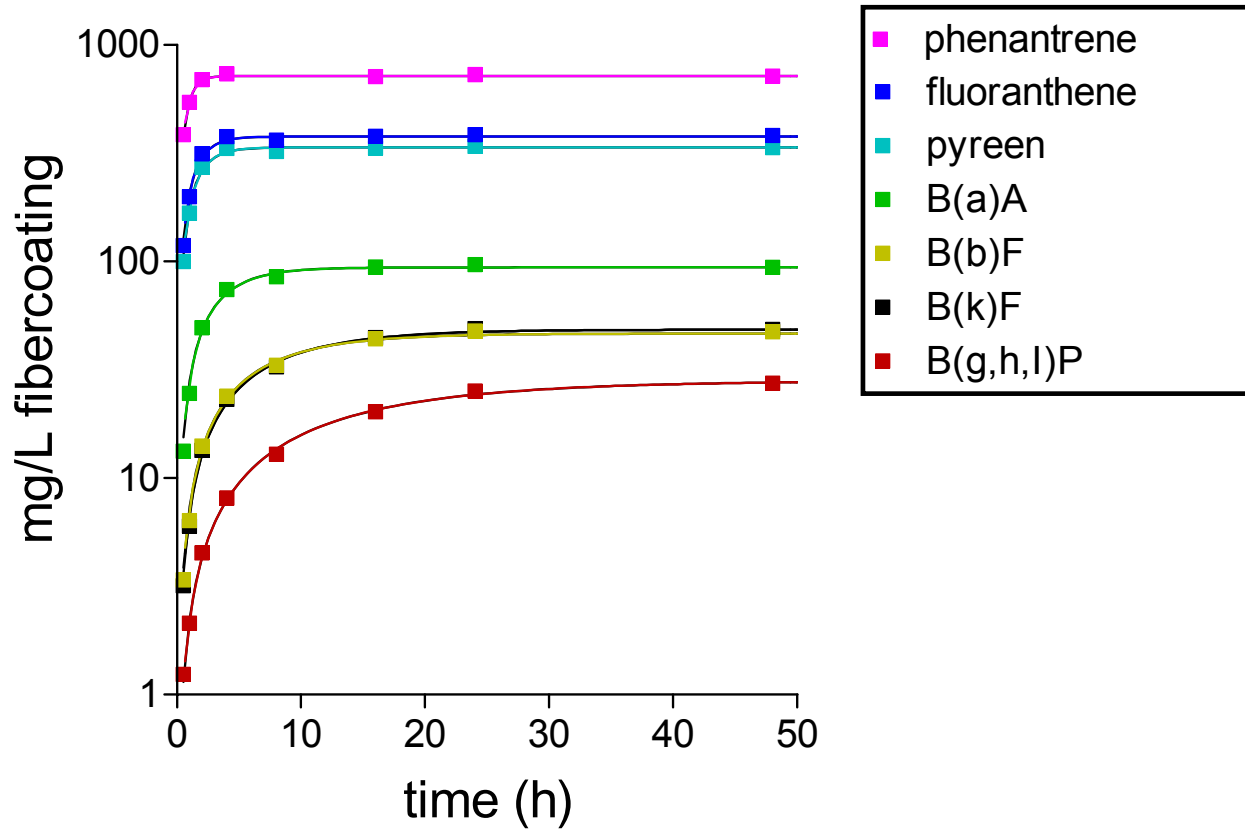
Assessing free concentrations of PAHs in soil



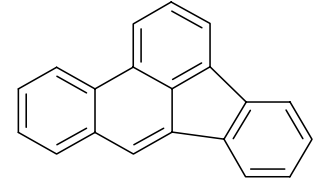
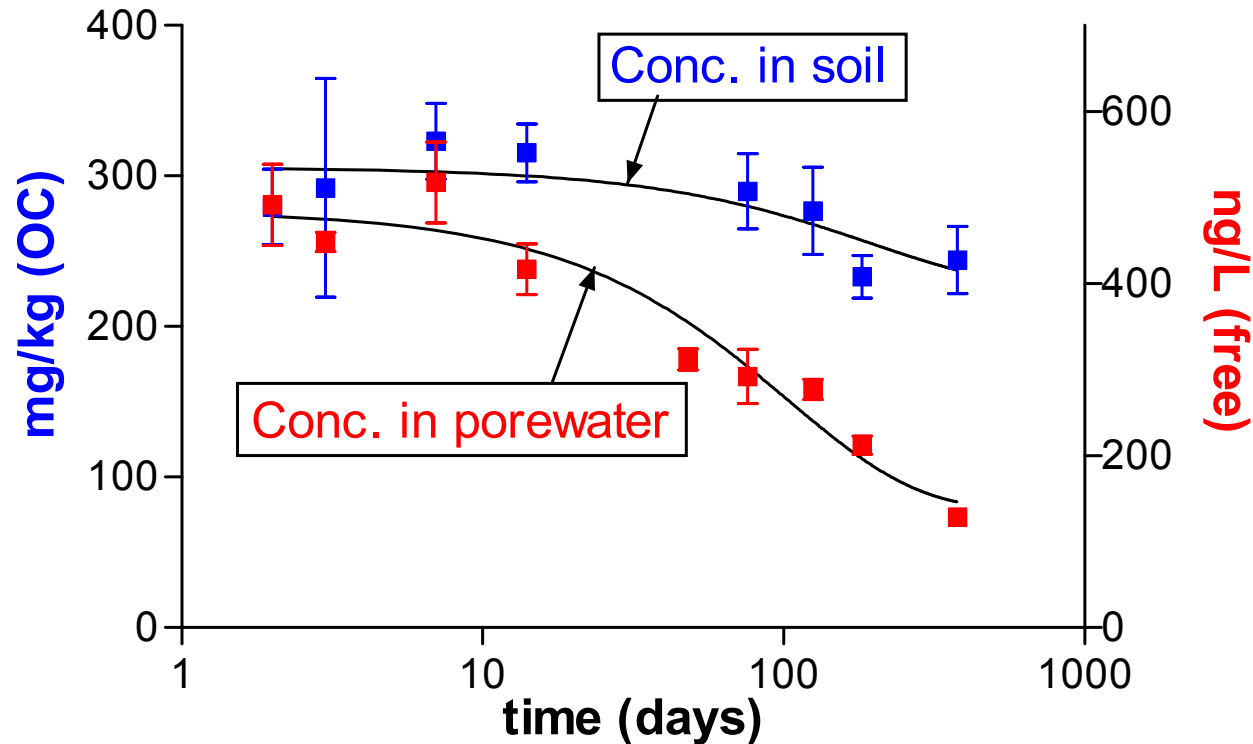
Assessing free concentrations of PAHs in soil



Kinetics of uptake into SPME fibers exposed to soil

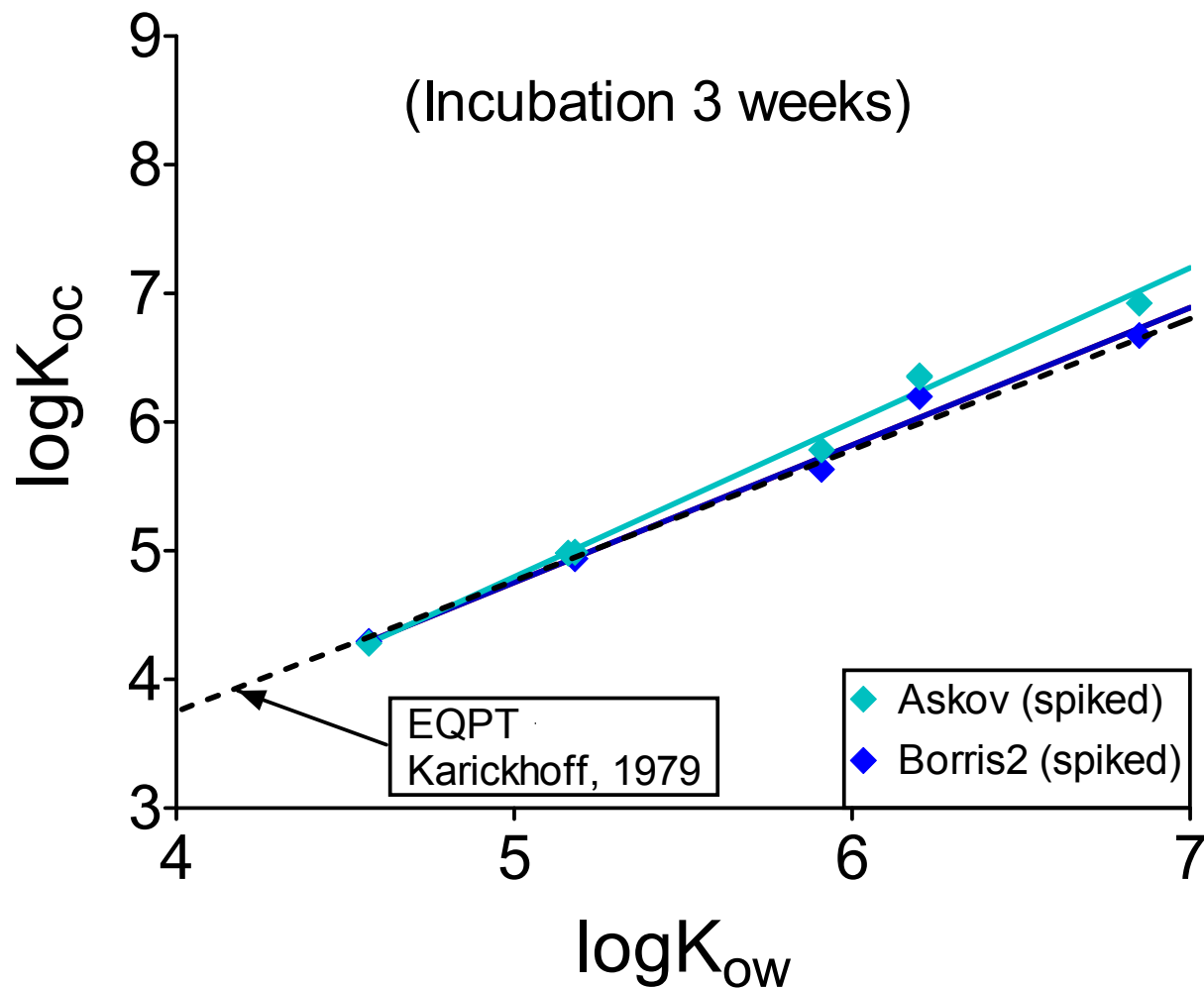


Aging of B[b]F in Askov soil

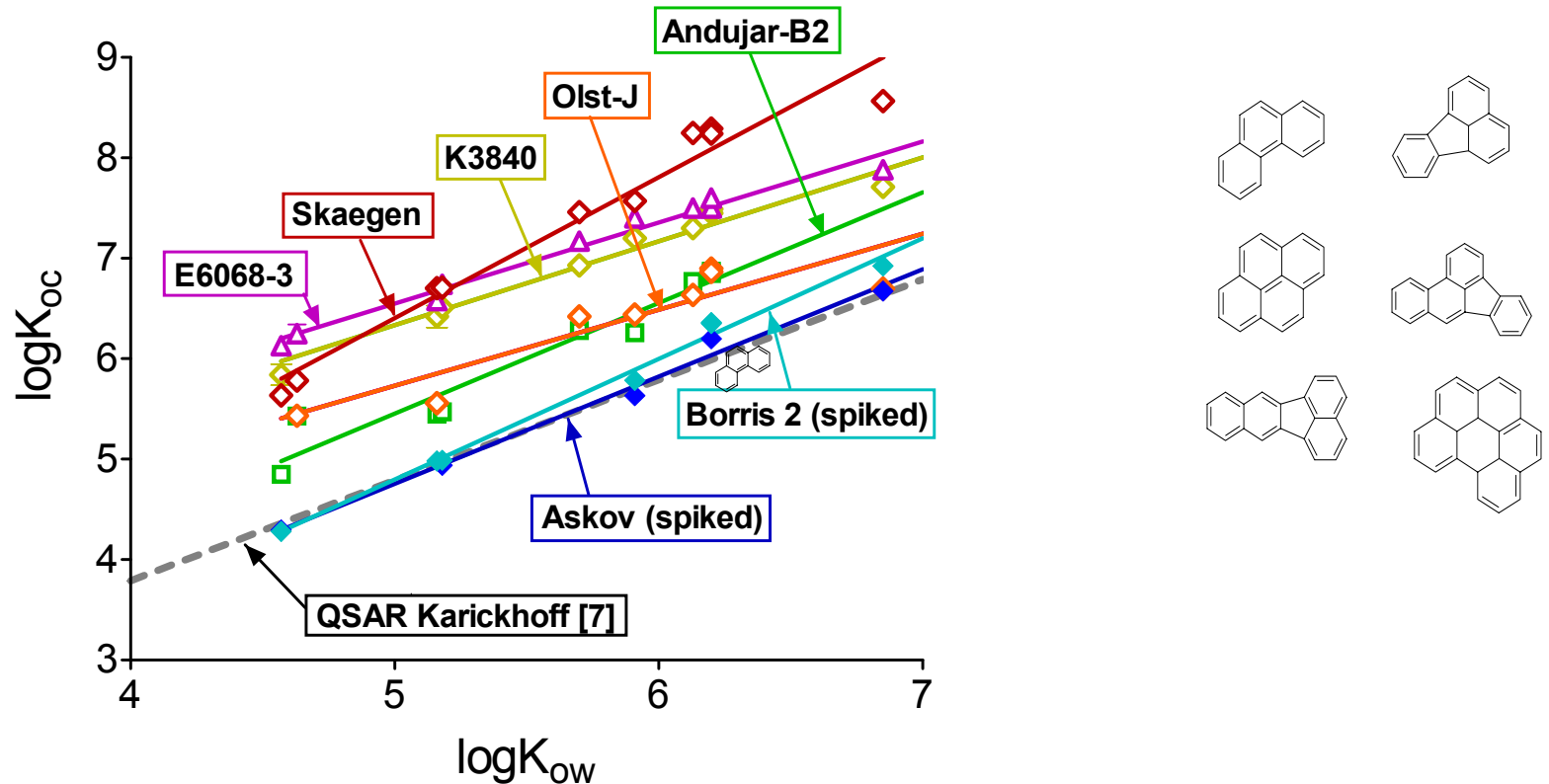


Aging reduces bioavailability, but maybe less than expected

Partition coefficients of PAHs in spiked soils



Partition coefficients of PAHs in spiked and field soils



Higher partition coefficients due to aging effects or presence of strongly binding components

Solid-Phase Microextraction To Predict Bioavailability and Accumulation of Organic Micropollutants in Terrestrial Organisms after Exposure to a Field-Contaminated Soil

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ROEL H. L. J. FLEUREN,[§]

ARJAN BARENDREGT,[†]

THEO L. SINNIGE,[†]

CORNELIS A. M. VAN GESTEL,[‡] AND

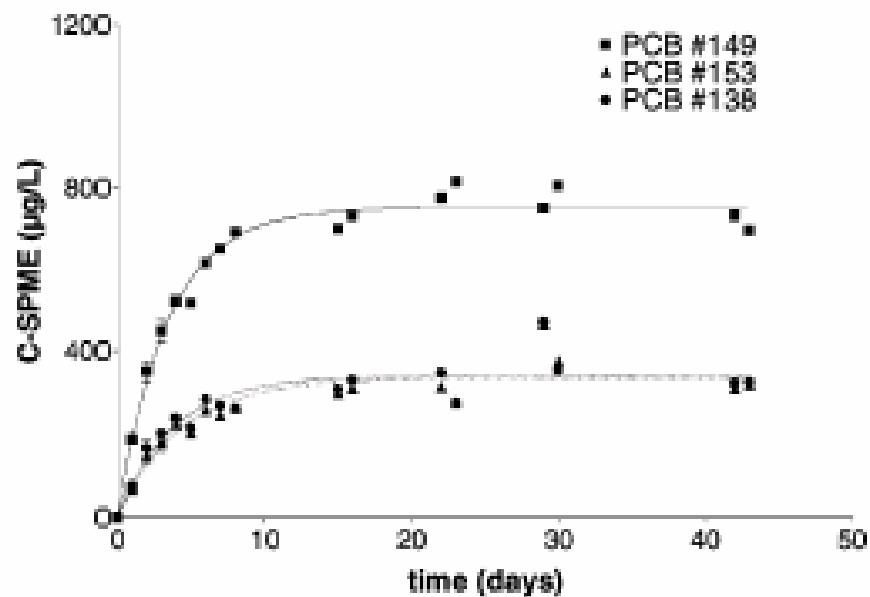
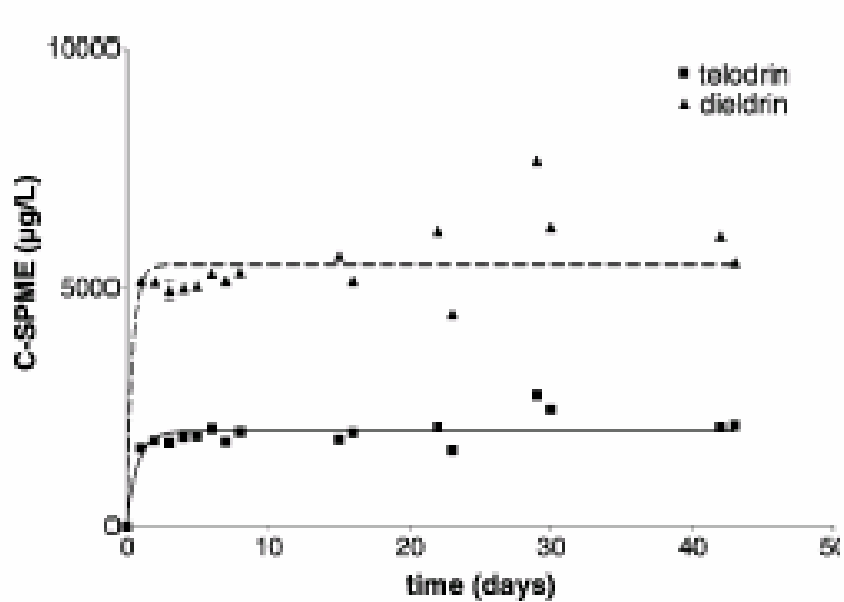
JOOP L. M. HERMENS[†]

Environ. Sci. Technol. 2004, 38, 4842-4848.

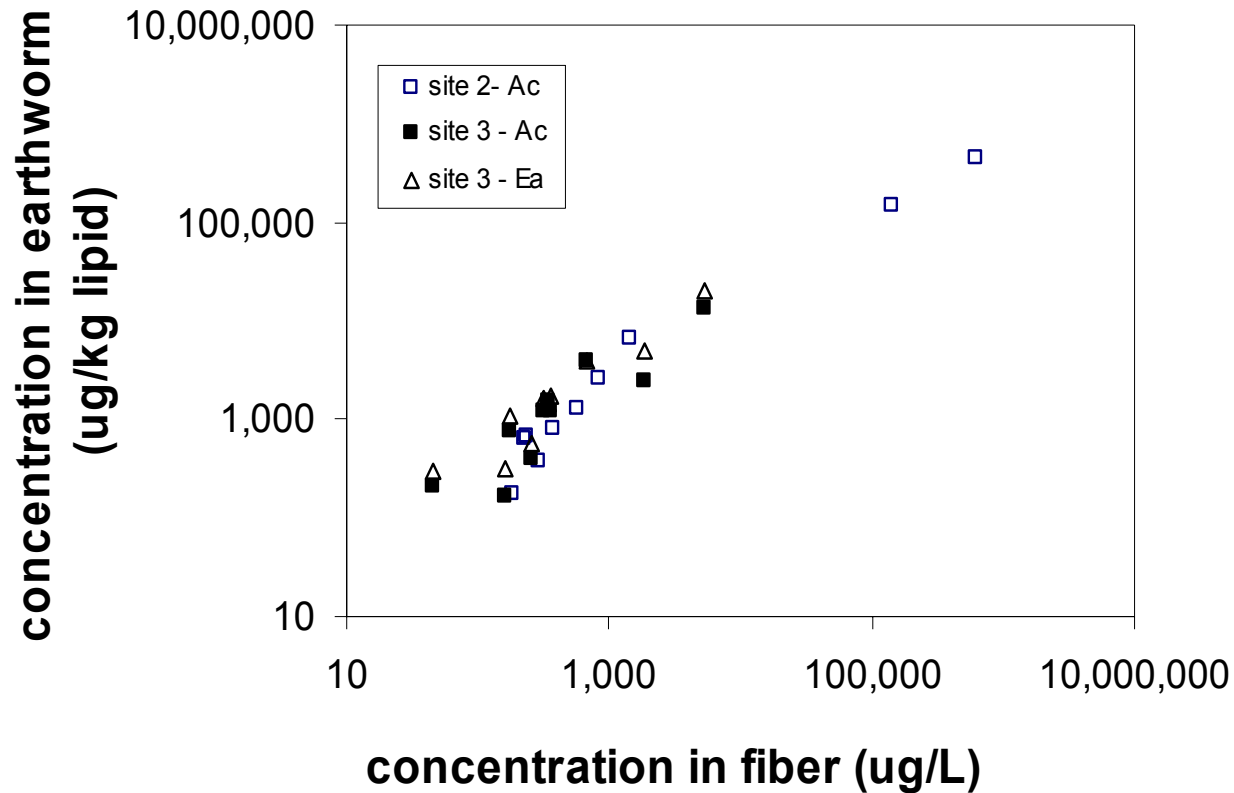
Application of nd-SPME to measure internal concentrations in soil organisms



Uptake in 30 μm PDMS coated fiber in a soil slurry



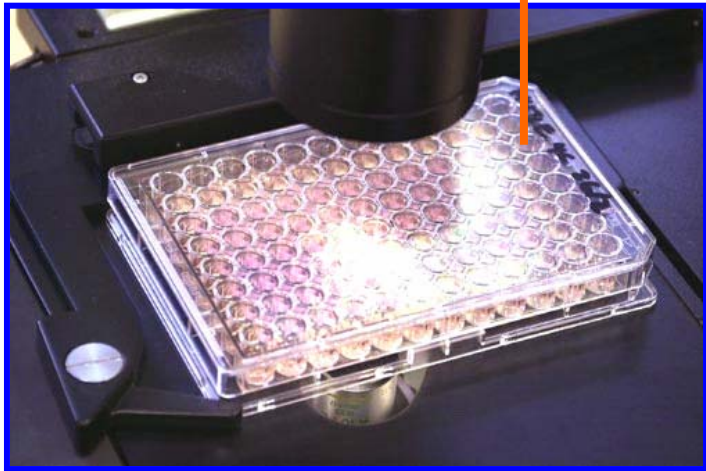
Application of nd-SPME to measure internal concentrations in soil organisms



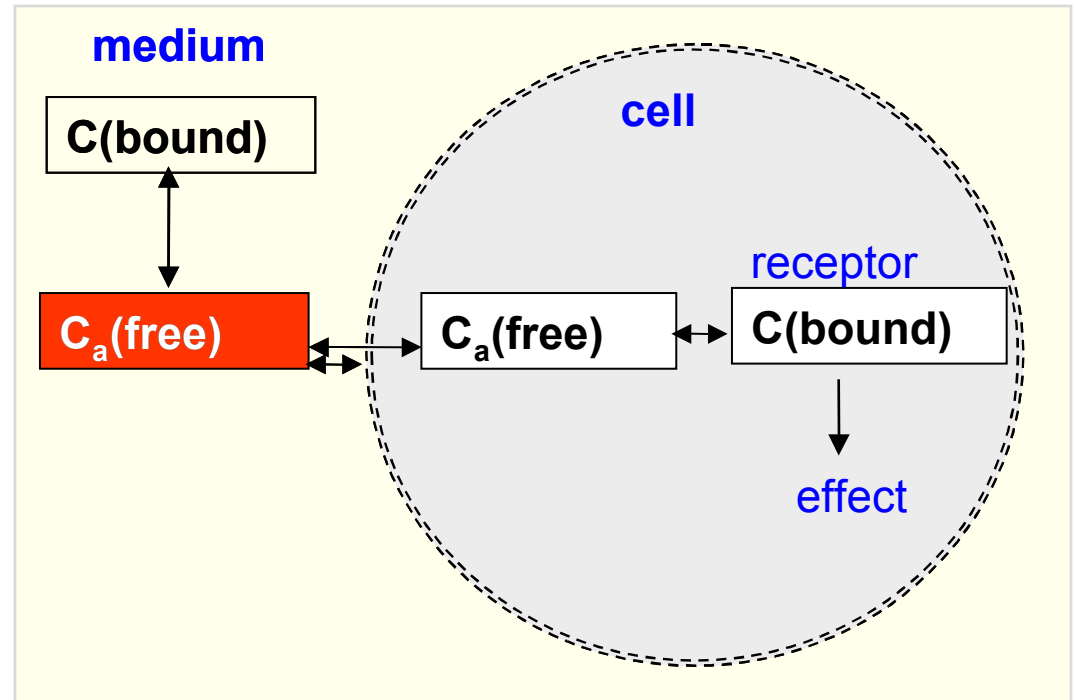
Application of solid phase micro-extraction to
measure freely dissolved concentration in *in vitro*
tests

PhD project: Minne Heringa (2004)

Exposure in *in vitro* tests

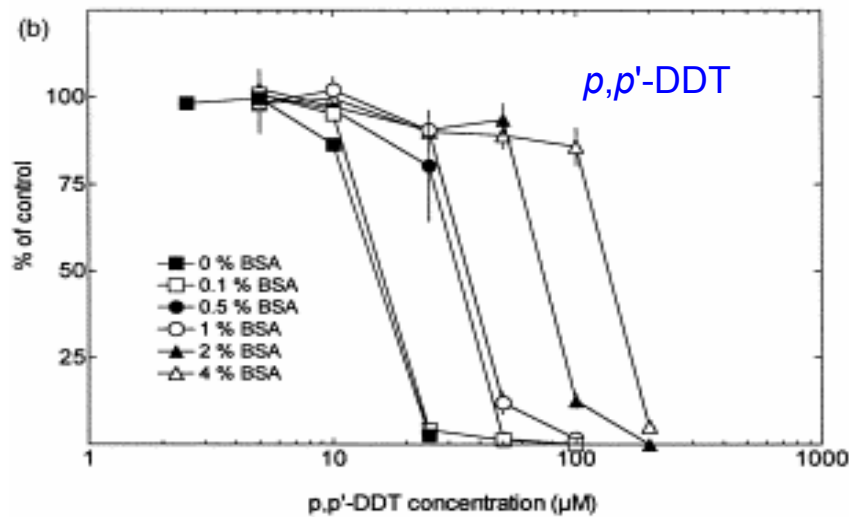


96 well plate



In vitro effect concentrations (nominal) will depend on medium

Influence of the medium albumin concentration on the concentration-effect relationships



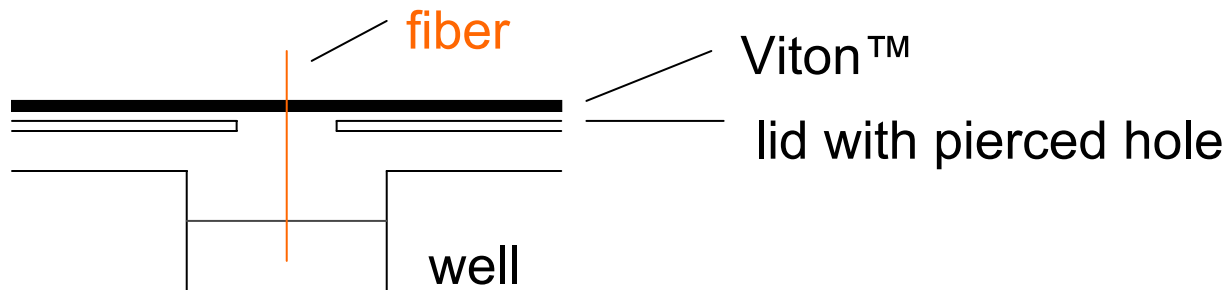
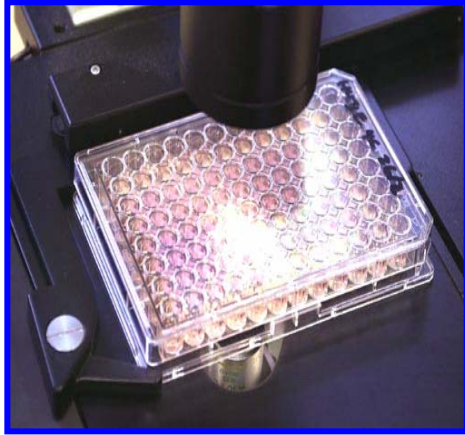
Nominal concentration

Application of nd-SPME to measure free concentrations in an *in vitro* assay for estrogenic activity

Experimental set-up

- estrogenicity reporter gene assay with 293 HEK cells with ER β (Hubrecht laboratory)
- 24-well plate
- Add compound, wait 24 hours
- Measure free concentration
- Test at different concentrations of serum in medium
- Establish dose-response curves for nominal and free concentrations

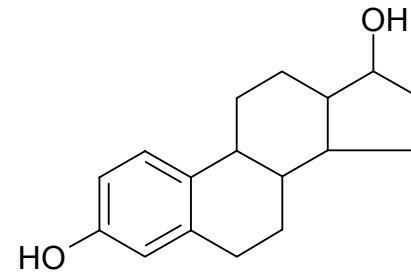
nd SPME applied in *in vitro* test for estrogenic activity performed in well plates



Compounds

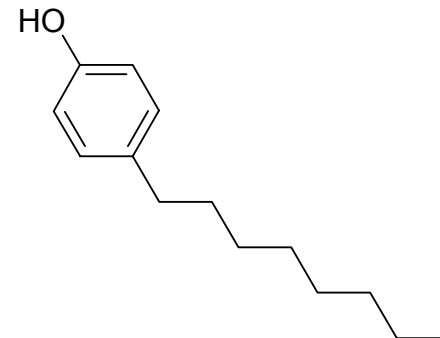
[³H]estradiol

- fibre coating: polyacrylate
- analysis: LSC

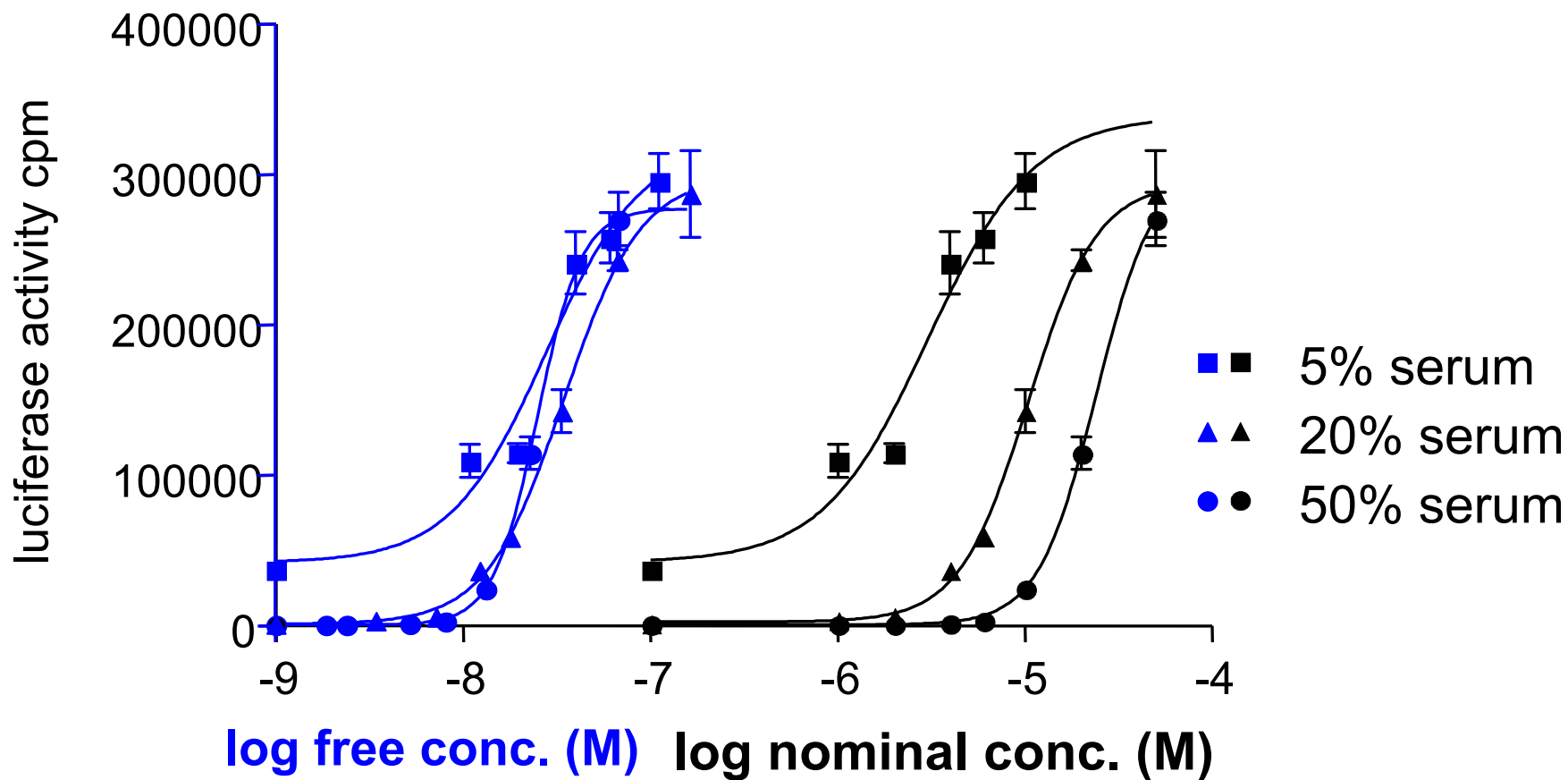


p-octylphenol

- fibre coating: PDMS
- analysis: GC-MS



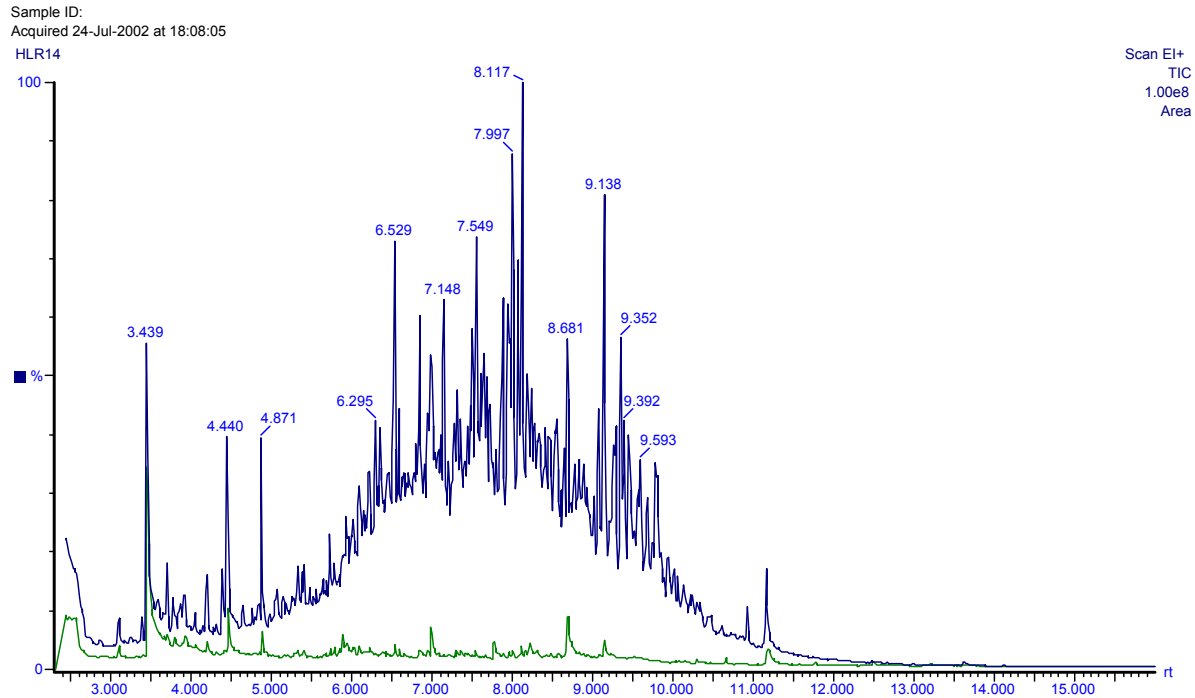
Results: nominal vs. free octylphenol concentration



Biomimetic solid phase microextraction method to estimate total body residue of petroleum products in sediment: a feasibility study

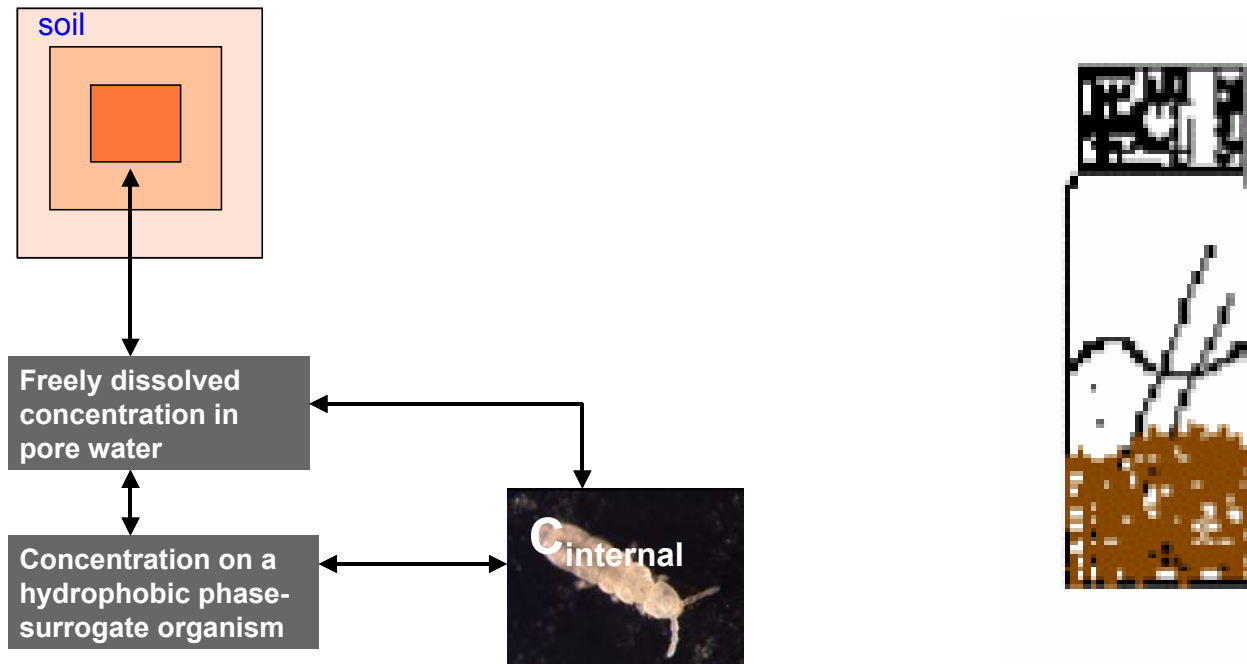
PhD project: Heather Leslie (2003)

Estimation of total body residue in an oil contaminated sediment



Full scan chromatogram of SPME fiber conditioned at 275 °C shown underneath a DMA peak of an exposed fiber in sediment containing spiked oil

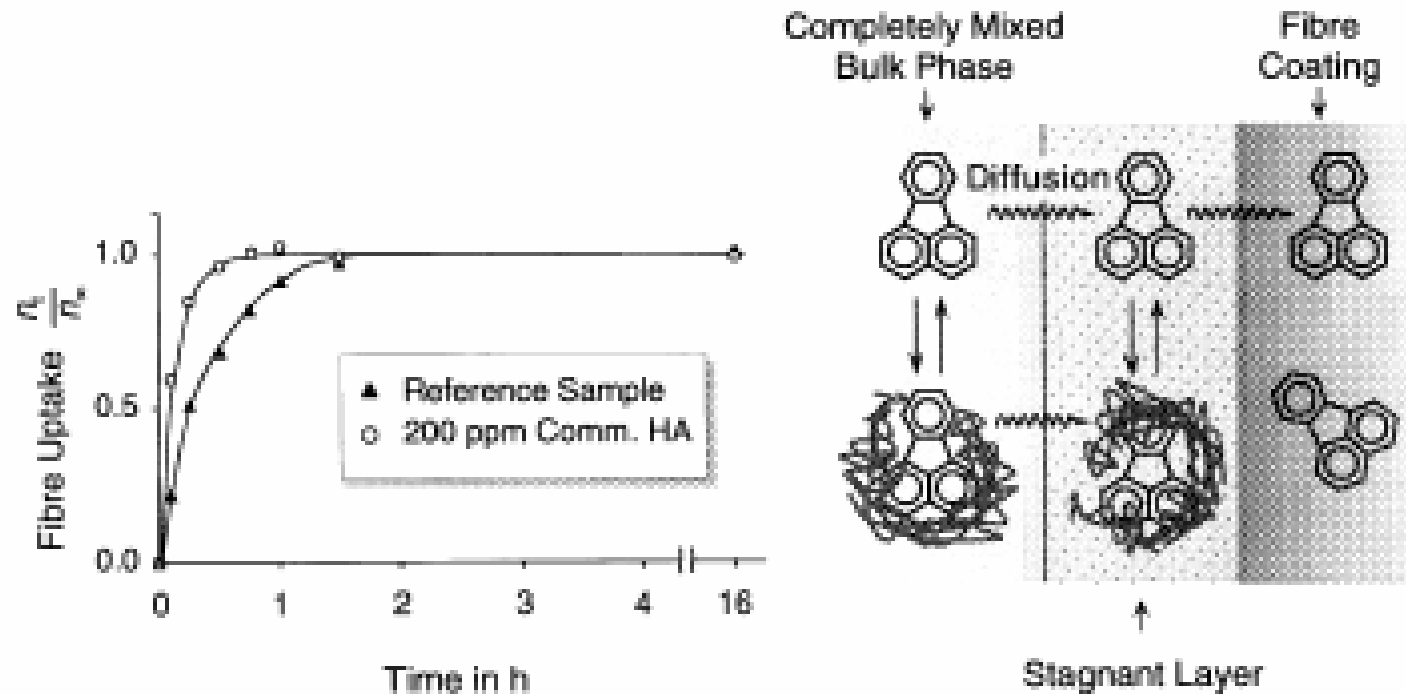
Nd SPME as method to measure “bioavailability”



Factors to consider in the analysis:

- Fouling of the SPME fiber
- Influence of matrix on uptake kinetics
- What are rate limiting steps in your system?
- Kinetic versus equilibrium measurements
- Calibration: for each chemical separately?

Nd SPME: potential influence of matrix on uptake kinetics



Oomen, A.G., Mayer, P. and Tolls, J. (2000). Anal. Chem. 72, 2802-

Heringa, M.B and Hermens, J.L.M. Trends in Analytical Chemistry, Vol. 22, No. 10, 2003, 575-597.

Characteristics of nd SPME

- non disturbing extraction: extraction does not affect the system
- direct measure of freely dissolved concentration
- measurement of site specific K_p values
- freely dissolved concentration is one step closer to an organism
- can be applied in miniature systems
- can be applied to measure partition coefficients to proteins, humic acids, soil and sediment, tissues etc.
- exposure time is long (equilibration times)
- does not give information about fraction “fast desorbing”

Additional slides

Experimental techniques for measuring free concentration and estimating internal dose

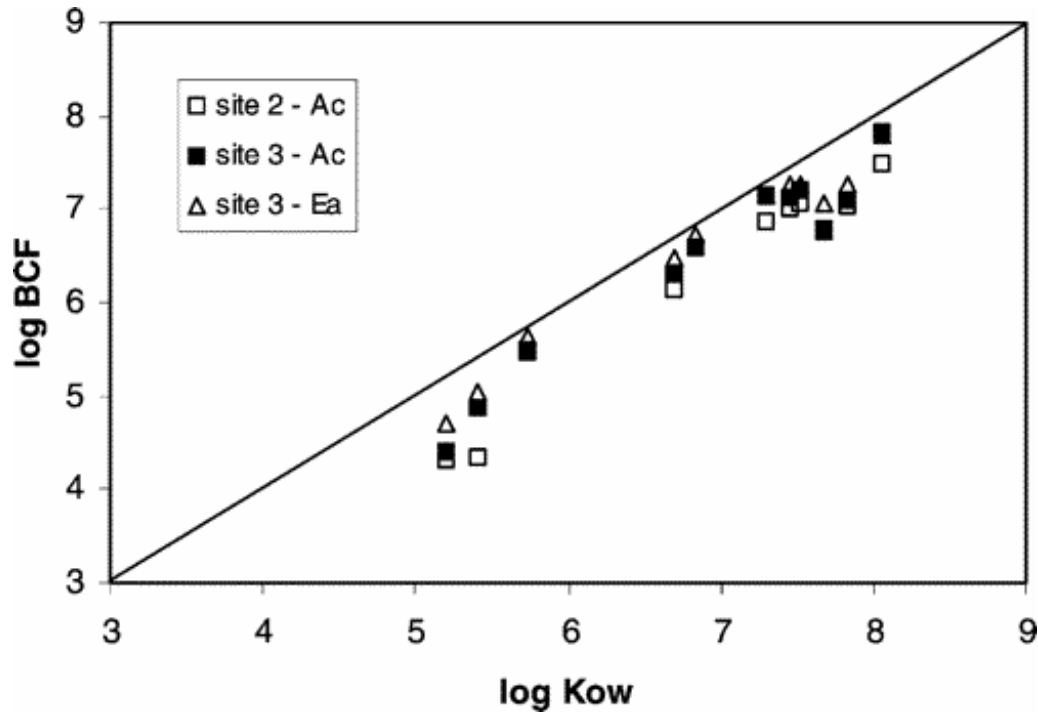
- equilibrium dialysis
- ultrafiltration and ultracentrifugation
- headspace analysis
- semi permeable membrane devices (SPMD) – Huckins, Sodergren
- solid phase partitioning (empore disk)
- thin polymer films on a glass plate
- **solid phase micro-extraction (SPME)**
- cyclodextrin extraction
- POM
- super critical fluid extraction (SFE)

External concentrations versus free and internal concentrations



Freely dissolved concentration is not the complete story, but it is an important aspect of the bioavailability issue.

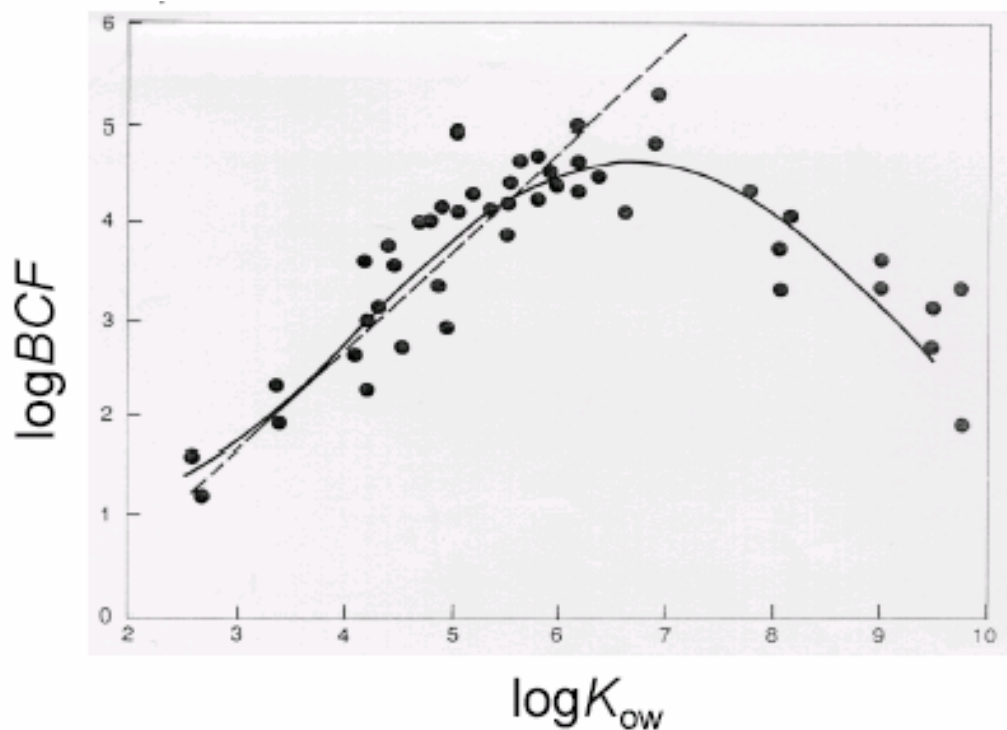
Relation between BCF and K_{ow} in earthworms



$$BCF = C_{\text{worm}} / C_{\text{pore-water}}$$

$C_{\text{pore-water}}$ measured via nd-SPME

Relation between BCF and K_{ow} : non linear



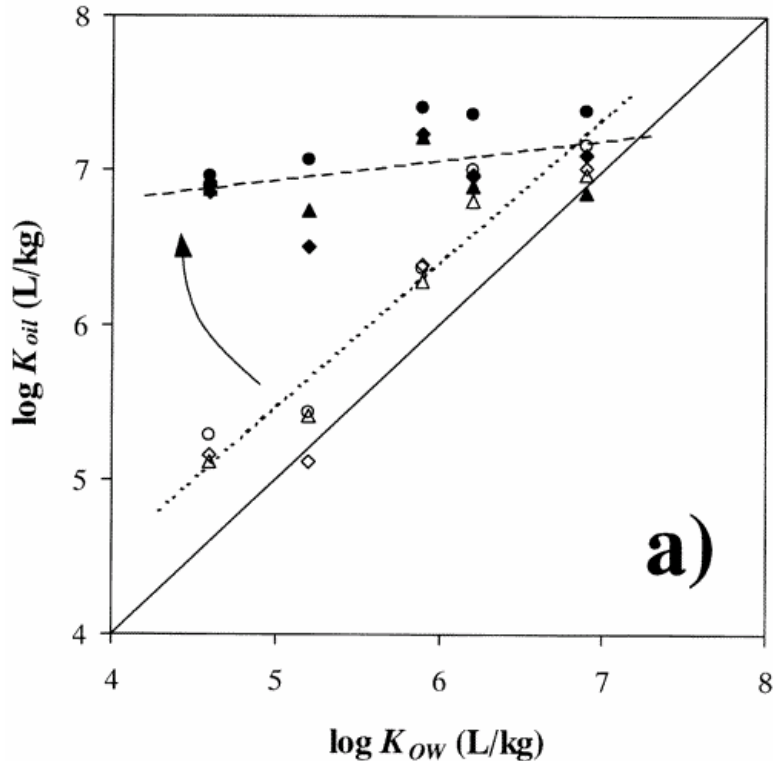
Gobas, F. A. C. P., Opperhuizen, A., Hutzinger, O. (1986), "Bioconcentration of hydrophobic chemicals in fish: relationship with membrane permeation", *Environ. Tox. Chem.* 5, 637-646.

Sorption of Polycyclic Aromatic Hydrocarbons to Oil Contaminated Sediment: Unresolved Complex?

MICHEL T. O. JONKER,^{*,†}
ANJA J. C. SINKE,[‡] JOS M. BRILS,[§] AND
ALBERT A. KOELMANS[†]

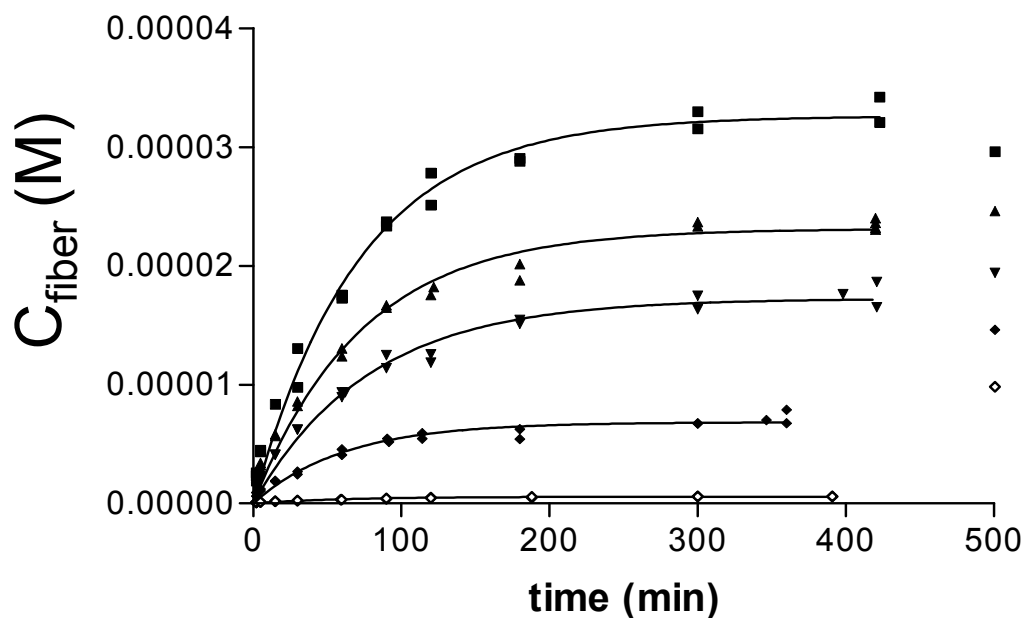
Environ. Sci. Technol. 2003, 37, 5197–5203

Effects of weathering on oil-water distribution

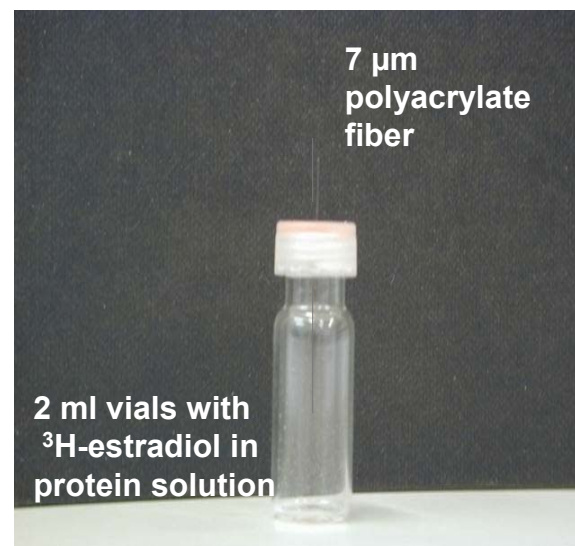


Effects of weathering on (a) oil-water distribution ($\log K_{oil}$) of PAHs. Open symbols are for unweathered oil/organic carbon; solid symbols are for weathered oil/organic carbon.

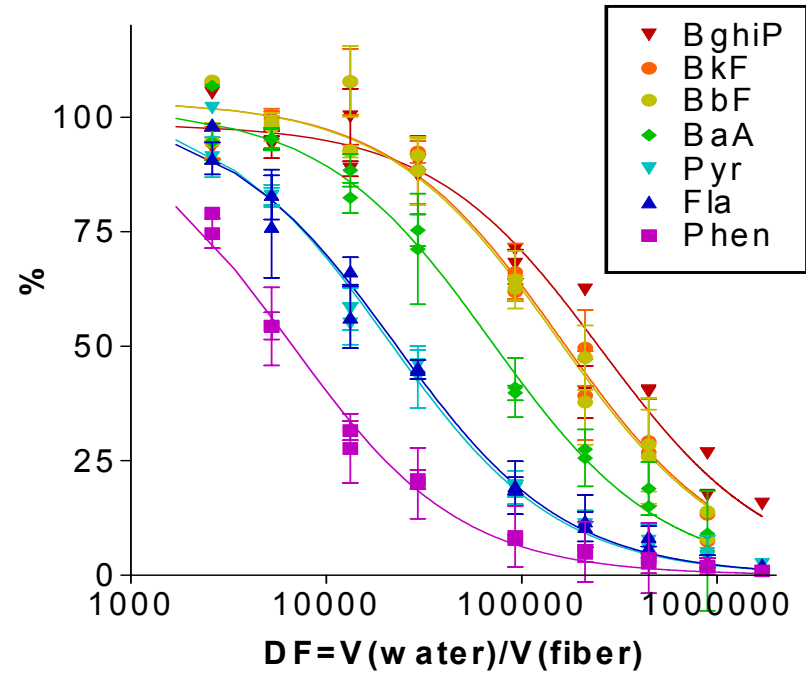
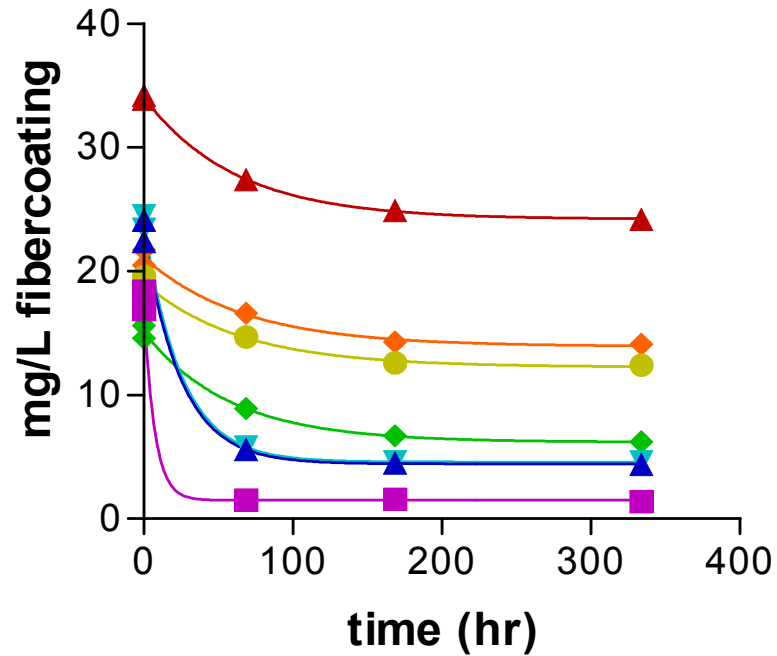
Uptake of estradiol to fiber at increasing protein concentration



- no protein
- ▲ 6.5×10^{-6} M BSA (ff=72%)
- ▼ 1.6×10^{-5} M BSA (ff=51%)
- ◆ 6.4×10^{-5} M BSA (ff=21%)
- ◇ 1.0×10^{-3} M BSA (ff=1.6%)



Measurement of fiber-water partition coefficients



$$\% fib_{(DF)} = \frac{\% fib_{(0)}}{\left(1 + \frac{DF}{K_P}\right)}$$

K fiber (PDMS) – water of PAHs

