COMBINING EQUILIBRIUM PASSIVE SAMPLING AND DOSING FOR THE MONITORING AND TOXICITY TESTING OF AQUATIC CONTAMINANT MIXTURES

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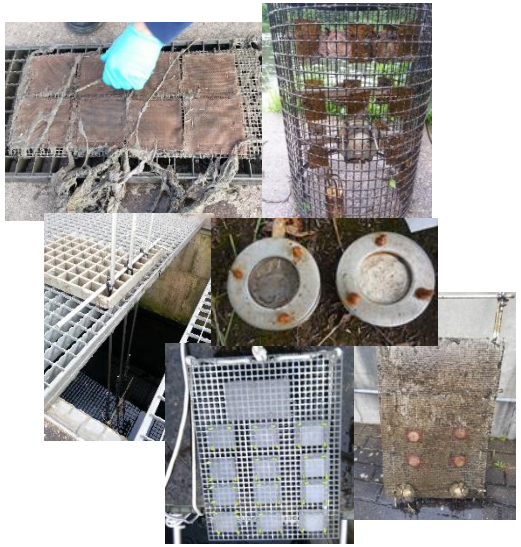
Passive sampling and mixture toxicity



Passive sampling for measuring mixture toxicity:

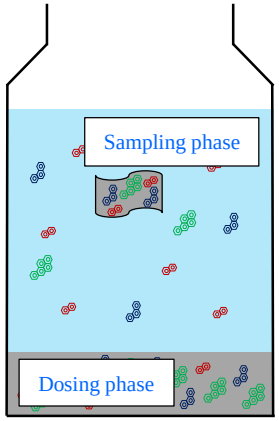
- *in situ* sampling in **different matrices** (e.g., water, sediment, soil)
- **complete mixture** profile sampled (even if not completely analyzed)
- **bioavailable dissolved levels** sampled

How to translate the sampler accumulated mixture back into the original bioavailable dissolved profile found in the field ?

Passive sampling and mixture toxicity

Medium (2% NaCl)

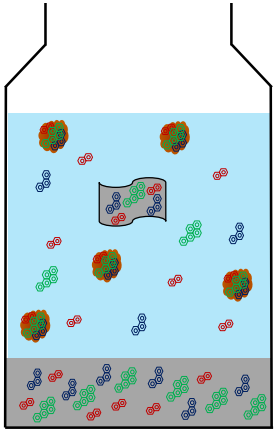
2 L



Sampling 1 to 175 h

Medium plus DOC

2 L



Sampling 1 to 175 h

Legend:

- Acenaphthene, phenanthrene, fluoranthene, benzo(a)pyrene
- Dissolved
- DOC sorbed
- Passive sampler sorbed (PDMS disk with 1cm diameter & 76 µm thickness)

Aqueous samples

- Extraction & spiking [1]

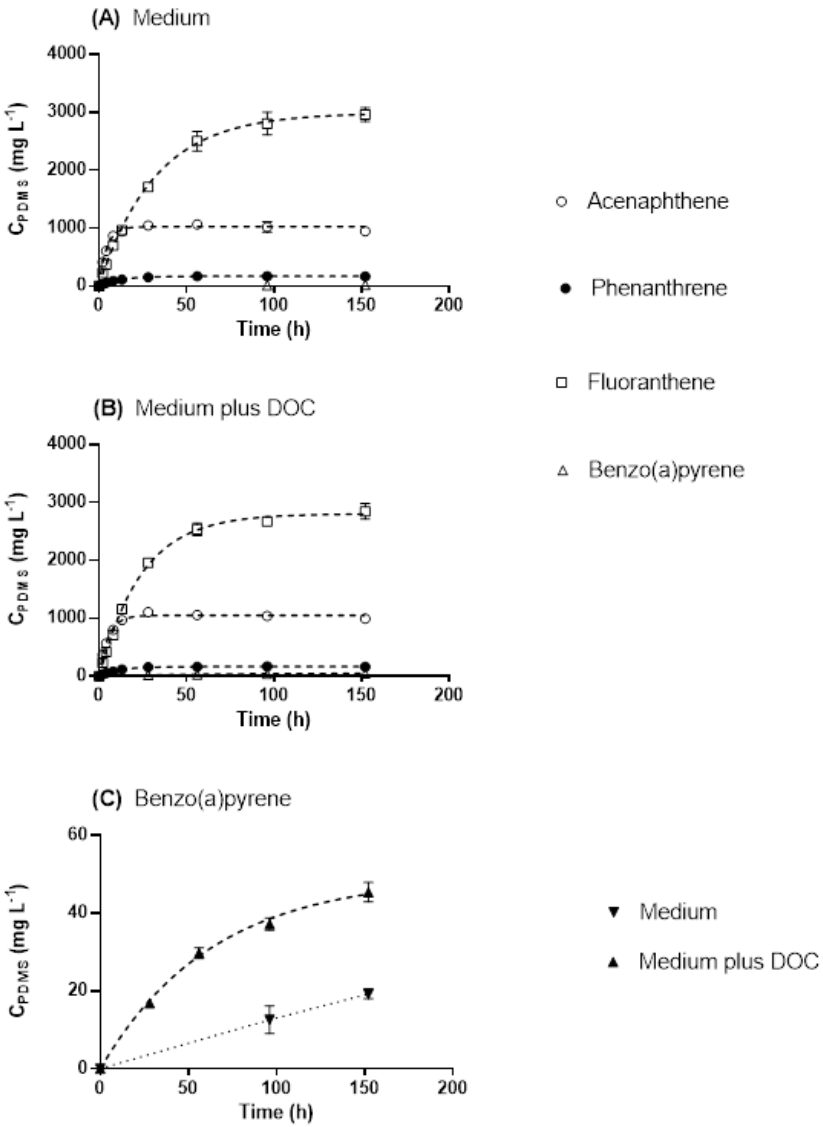
Passive samplers

- Extraction & spiking [2]
- Passive dosing

[1] 5mL aqueous sample extracted, transferred into 50 µL DMSO, 10 µL spiked into test

[2] One disk extracted, transferred into 50 µL DMSO, 10 µL spiked into test

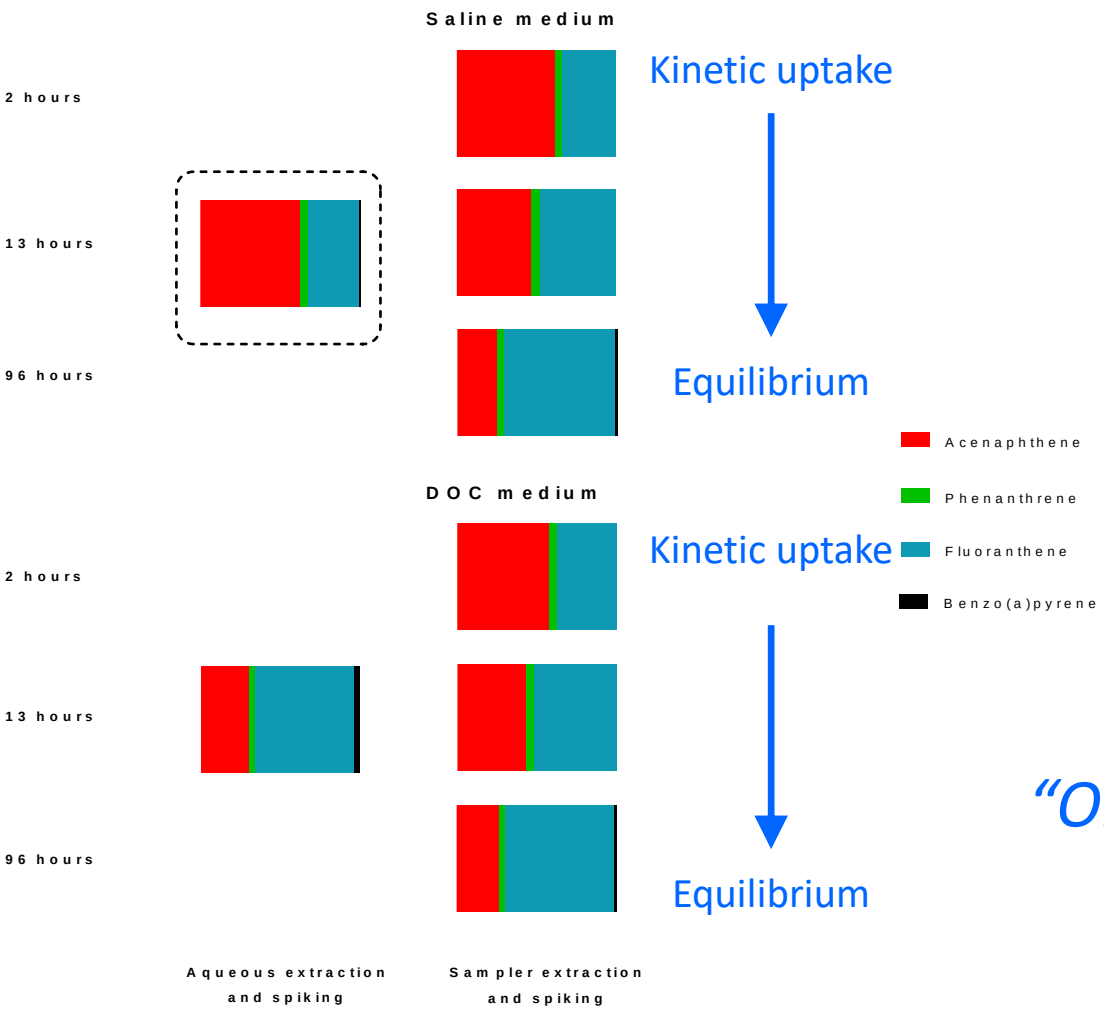
Passive sampling and mixture toxicity



Typical passive sampler uptake

- less hydrophobic PAHs faster equilibration
- more hydrophobic PAHs slower equilibration
- DOC increasing uptake kinetics of hydrophobic PAHs (enhanced diffusion)

Sampler extraction and spiking



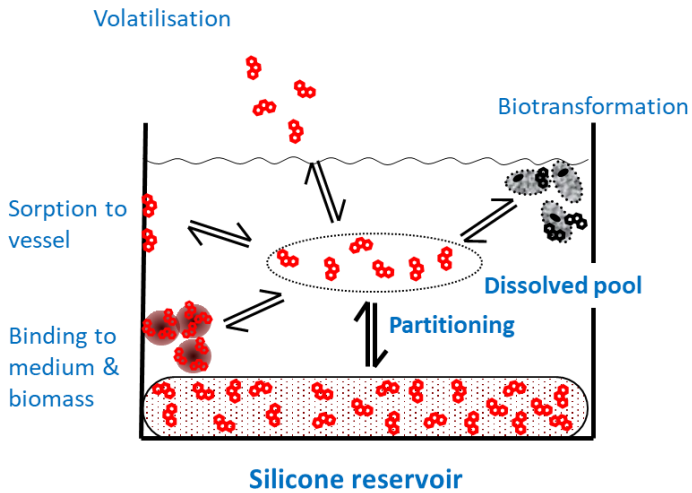
- spiked mixture reflecting sampler kinetics
- initially dominated by lighter PAHs
- later dominated by heavier PAHs as approach equilibrium

"Once skewed always skewed"

Passive sampler extraction and spiking

Solvent spiking anyhow results in poor exposure control

Passive dosing and exposure control



Exposure concentration: $$C_{Free} = \frac{C_{Polymer}}{K_{Polymer/Free}}$$

- no solvent
- control C_{free} and not C_{total}
- defined and constant C_{free}
- analytical advantages

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DOI: 10.1002/etc.453

DEVELOPMENT OF A POLYDIMETHYLSILOXANE FILM-BASED PASSIVE DOSING METHOD IN THE IN VITRO DR-CALUX® ASSAY

Petra Booij,*† Marja H. Lamoree,† Pim E.G. Leonards,† Peter H. Cenijn,† Hans J.C. Klamer,‡ L. Alexander van Vliet,‡ Johan Åkerman,‡ and Juliette Legler†
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(Submitted 7 April 2010; Returned for Revision 12 May 2010; Accepted 20 October 2010)

Environmental Science & Technology
Article
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Assessing Aromatic-Hydrocarbon Toxicity to Fish Early Life Stages Using Passive-Dosing Methods and Target-Lipid and Chemical-Activity Models

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MEASURING THE TOXICITY OF ALKYL-PHENANTHRENES TO EARLY LIFE STAGES OF MEDAKA (*ORYZIAS LATIPES*) USING PARTITION-CONTROLLED DELIVERY

Dominique Turcotte, Parveen Akhtar, Michelle Bowerman, Yiannis Kiparissis, R. Stephen Brown, and Peter V. Hodson*
Queen's University, Kingston, Ontario, Canada

(Submitted 20 May 2010; Returned for Revision 19 June 2010; Accepted 22 September 2010)

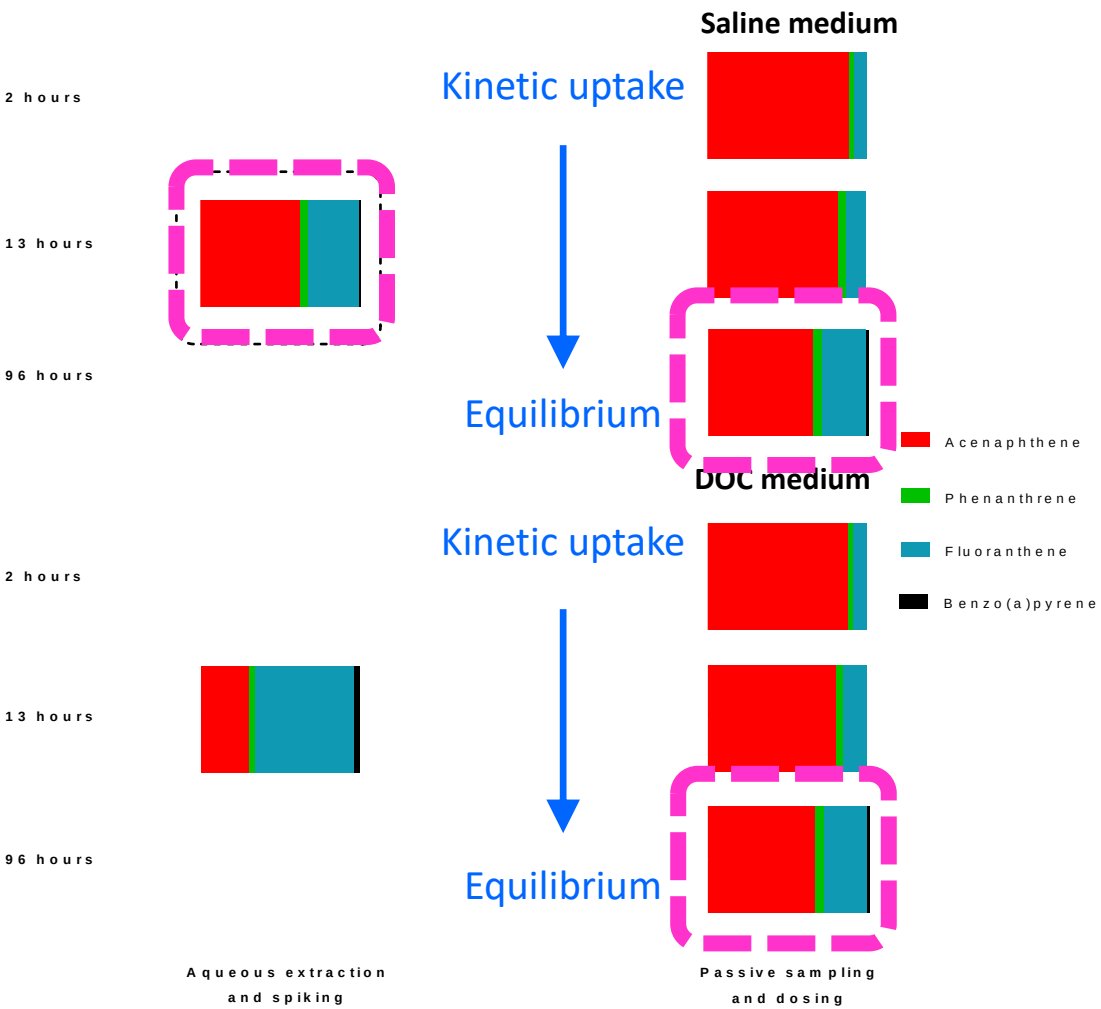
Environmental Science & Technology
Article
pubs.org/est

Changes in the Expression of *cyp35a family* Genes in the Soil Nematode *Caenorhabditis elegans* under Controlled Exposure to Chlorpyrifos Using Passive Dosing

Ji-Yeon Roh, Hwang Lee, and Jung-Hwan Kwon*
Division of Environmental Science and Ecological Engineering, Korea University, 145 Anam-ro, Seongbuk-gu, Seoul 136-713, Republic of Korea

plus others .....

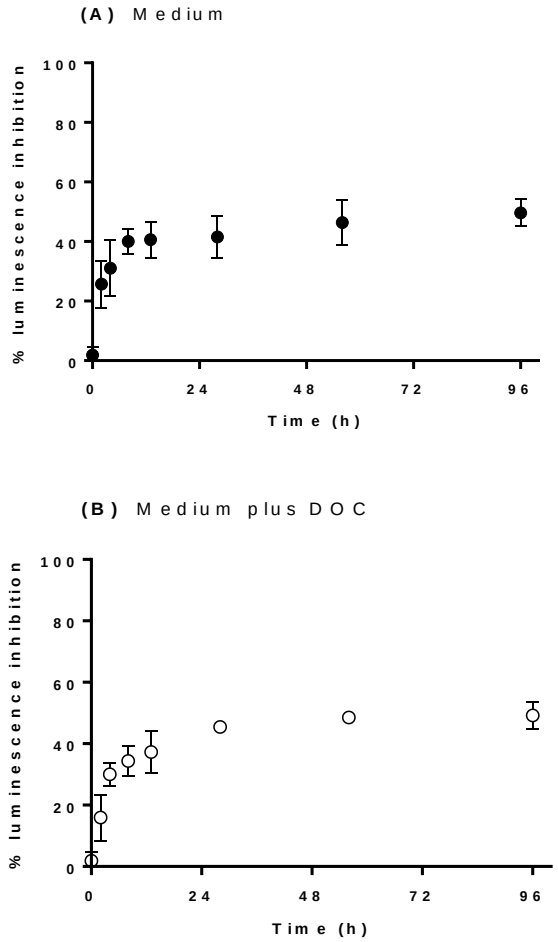
Passive sampling and dosing



- dosed mixture reflecting sampler uptake kinetics
- initially dominated by lighter PAHs
- equilibrium passive sampling and dosing recreating original mixture

Passive sampling and dosing

Mixture toxicity in Microtox bioassay



- reflected in measured mixture toxicity
- initially passively dosed mixture wrong profile and too low levels
- later passively dosed mixture correct profile and levels
- same mixture toxicity in set-ups with and without DOC (= same bioavailable dissolved profile !!)

Literature on equilibrium sampling and dosing

Marine Pollution Bulletin 93 (2015) 9–19

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Passive sampling reversed: Coupling passive field sampling with passive lab dosing to assess the ecotoxicity of mixtures present in the marine environment

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Environmental Science & Technology

Feature

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Strategies for Transferring Mixtures of Organic Contaminants from Aquatic Environments into Bioassays

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Transferring mixtures of chemicals from sediment to a bioassay using silicone-based passive sampling and dosing†

Lukas Mustajärvi,*[a] Ann-Kristin Eriksson-Wiklund,[a] Elena Gorokhova,[a] Annika Jahnke[b] and Anna Sobek[a]

Environmental mixtures of chemicals consist of a countless number of compounds with unknown identity and quantity. Yet, chemical regulation is mainly built around the assessment of single chemicals. Existing frameworks for assessing the toxicity of mixtures require that both the chemical composition and quantity are known. Quantitative analyses of the chemical composition of environmental mixtures are however extremely challenging and resource-demanding. Bioassays may therefore serve as a useful approach for investigating the combined toxicity of environmental mixtures of chemicals in a cost-efficient and holistic manner. In this study, an unknown environmental mixture of bioavailable semi-hydrophobic to hydrophobic chemicals was sampled from a contaminated sediment in a coastal Baltic Sea area using silicone polydimethylsiloxane (PDMS) as an equilibrium passive sampler. The chemical mixture was transferred to a PDMS-based passive dosing system, and its applicability was demonstrated using green algae *Tetraselmis suecica* in a cell viability assay. The proportion of dead cells increased significantly with increasing exposure level and in a dose–response manner. At an ambient concentration, the proportion of dead cells in the population was nearly doubled compared to the control; however, the difference was non-significant due to high inter-replicate variability and a low number of replicates. The validation of the test system regarding equilibrium sampling, loading efficiency into the passive dosing polymer, stability of the mixture composition, and low algal mortality in control treatments demonstrates that combining equilibrium passive sampling and passive dosing is a promising tool for investigating the toxicity of bioavailable semi-hydrophobic and hydrophobic chemicals in complex environmental mixtures.

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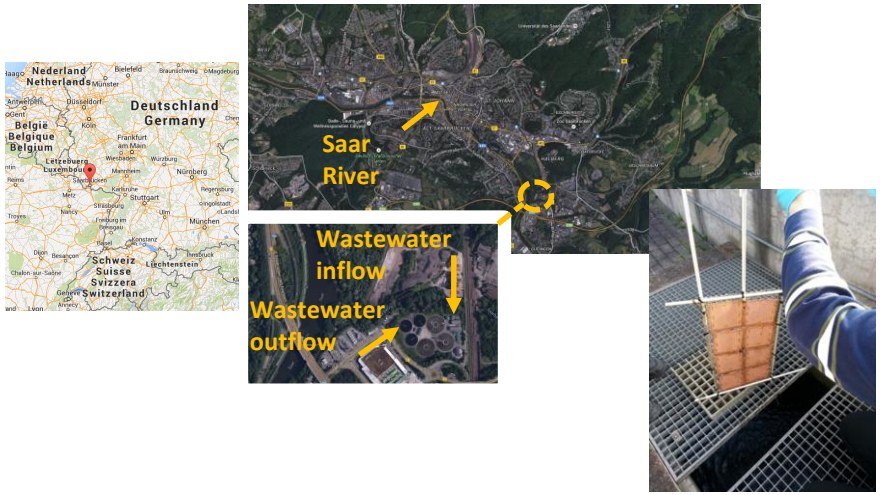
rsc.li/espi

Equilibrium passive sampling and dosing using silicone

Equilibrium sampling and dosing in surface water



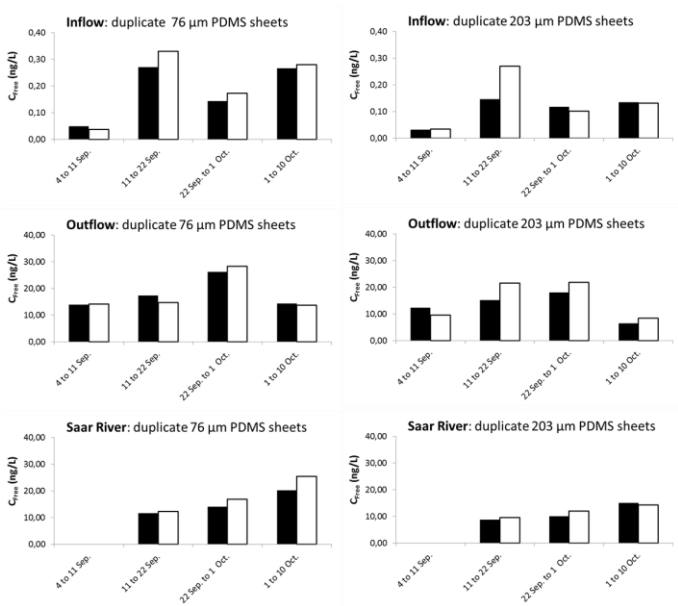
Passive samplers in inflow and outflow of the Brebach wastewater treatment plant (WWTP) as well as the receiving Saar River

Passive samplers 10 x 10 cm PDMS silicone sheets with thicknesses of either 76 or 203 µm

Exchanged at ca. 10 day intervals

LC-MS/MS analysis of 37 biocides, 6 pharmaceuticals and 4 industrial chemicals

Equilibrium or not?



Isoproturon as an example

Good agreement observed between duplicate passive samplers of each thickness

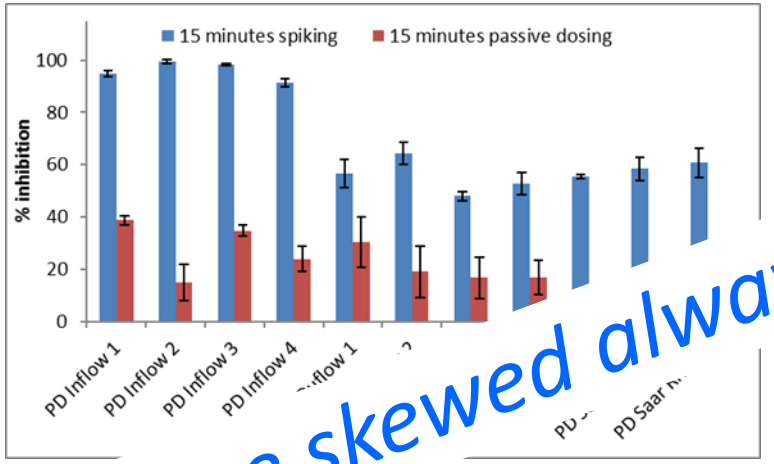
203 µm passive samplers slightly lower concentrations indicating partitioning equilibrium had not been fully reached in thicker disks

Applies to polar compounds only !

Equilibrium sampling and dosing in surface water

Toxicity testing at environmental levels

- **Equilibrium passive sampling and dosing:** PDMS disks introduced directly into bioassay
- **Solvent spiking :** PDMS disk extracted in methanol, exchanged into DMSO and spiked into bioassay



Mi an example

"Once skewed always skewed"

Sampler extr nd spiking higher mixture toxicity compared to passive dosing

- Contaminants up-concentration in polymer offsetting lack of buffering with spiking.

Equilibrium sampling and dosing in surface water

Does this approach using silicone correctly measure environmental mixture toxicity?

NO !!

- more hydrophobic compounds not reaching a partitioning equilibrium during passive sampling *(= under-represented in mixture toxicity*)
- more polar compounds not adequately buffered during passive dosing *(= under-represented in mixture toxicty)*

The importance of polymer affinity

Silicones great for passive sampling of certain compounds

Silicones also most common passive dosing pase:

- practical
- biocompatible
- low internal resistance (no internal gradients)
- required affinity for hydrophobic compounds

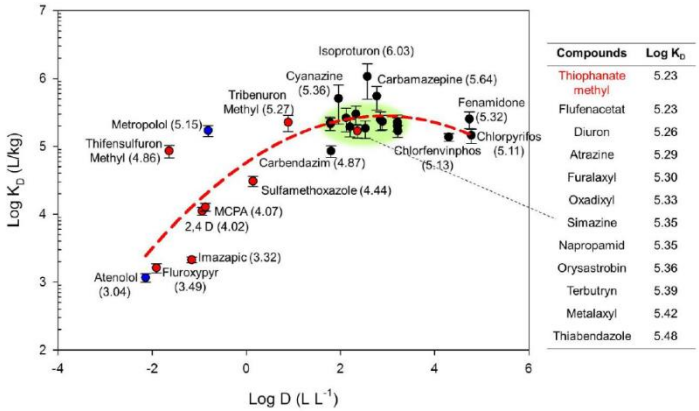
Do not have high enough affinity for more polar contaminants

Stability of concentration in polymer during passive dosing critical

$$C_{Free} = \frac{C_{Polymer}}{K_{Polymer/Free}}$$

What about a mixed polymer?

For example, silicone plus SPE polymers



Compounds	Log K_D
Thiophanate methyl	5.23
Flufenacetat	5.23
Diuron	5.26
Atrazine	5.29
Furalaxyl	5.30
Oxadixyl	5.33
Simazine	5.35
Napropamid	5.35
Orysastrobin	5.36
Terbutryn	5.39
Metalaxyl	5.42
Thiabendazole	5.48

Partioning to OASIS HLB®

Jeong et al. Chemosphere 174 (2017) 297- 305.

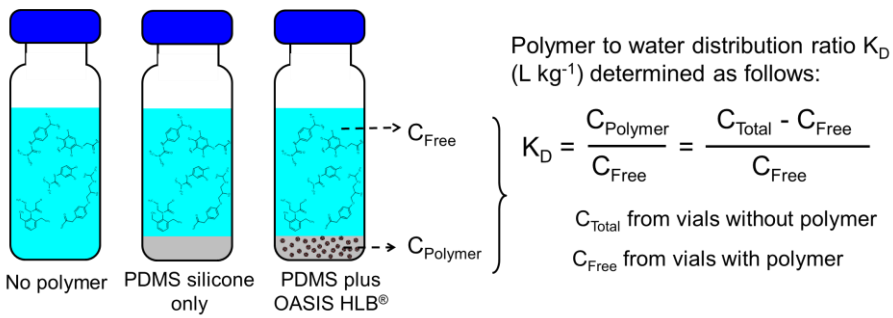
Pros

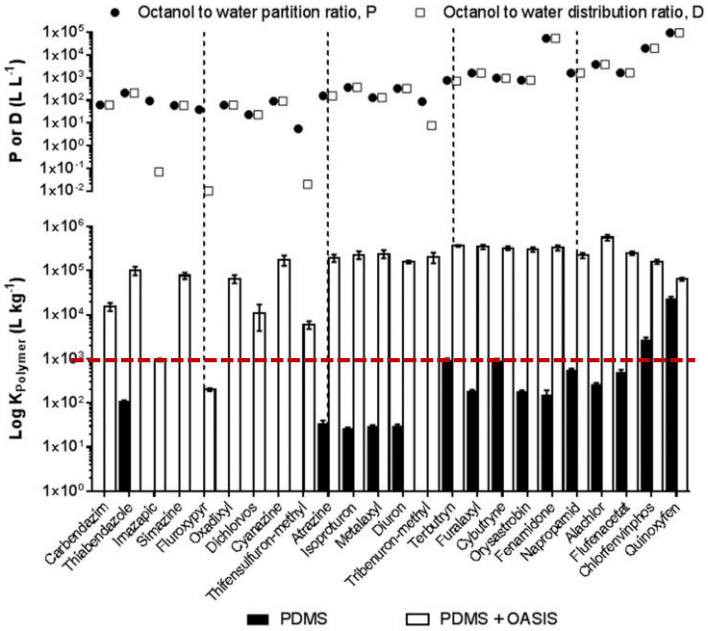
- pragmatic solution that can immediately applied
- in-house making of different formats (disks, coatings etc.)
- can be customised by varying SPE polymer types and amounts
- low ionization aretfacts in ESI

Cons

- complicated (main resistance to uptake? how does the silicone matrix affect SPE polymer sorption?)
- sorption non-linear under certain conditions (e.g., mixtures, high concentrations)

Development of a mixed polymer

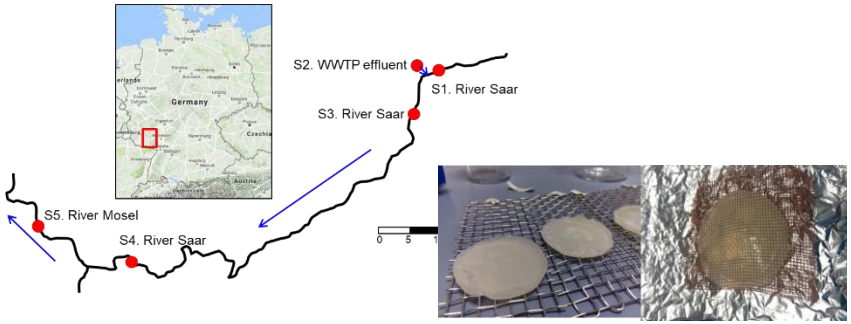




- Mixed polymer consisting of PDMS silicone embedded with 30 µm Oasis HLB® particles
- PDMS silicone (1000 – 0.001 µg, steps of 10) was used as a reference
- 1 mL of pH7 buffer added and spiked with a mixture of 24 compounds
- Equilibrated before HPLC-MS/MS analysis

- Data for 1000 µg OASIS HLB® in 1000 µg PDMS
- Mixed polymer marked increased affinity
- Suitable for passive sampling and dosing?

Mixed polymer passive sampling in waters



POCIS (integrative) and Mixed Polymer Sampler (equilibrium) deployed in parallel

Similar range of compounds detected (including polar compounds)

Calculated dissolved concentrations similar (especially considering range in POCIS sampling rates)

Mixed polymer sampler lower ionization suppression in ESI source of LC-MS/MS

Jeong et al. Submitted.

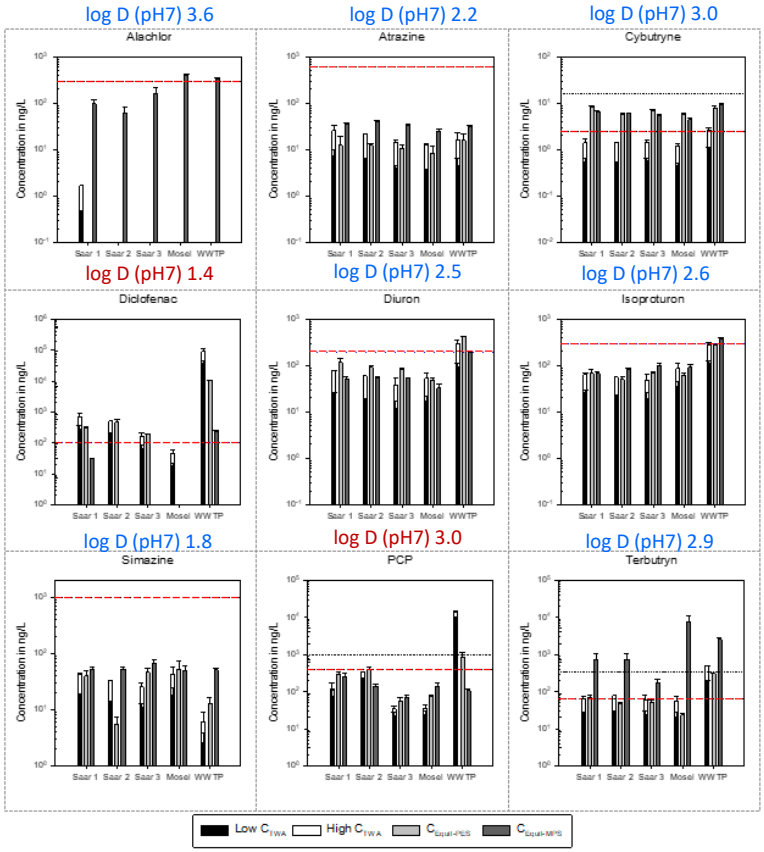


Figure 3. Dissolved concentrations of the 8 priority substances and diclofenac at the five sampling sites expressed as a low and high C_{TWA} from the POCIS Oasis HLB measurements (black and white bar), $C_{Equil\text{-}PES}$ from the POCIS PES membranes (light gray bar) and $C_{Equil\text{-}MPS}$ from the MPS (dark gray bar). The red dashed lines represent the AA-EQS and the black dotted lines the MAC-EQS. Error bars stand for the standard error of the mean.

Black + white bar = POCIS Dark grey bar = Mixed sampler

Direct passive dosing using OASIS HLB?

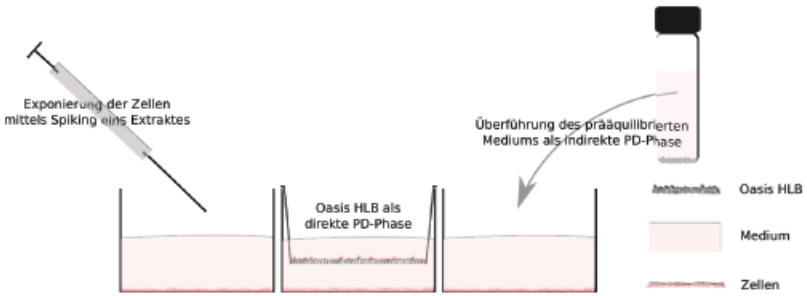
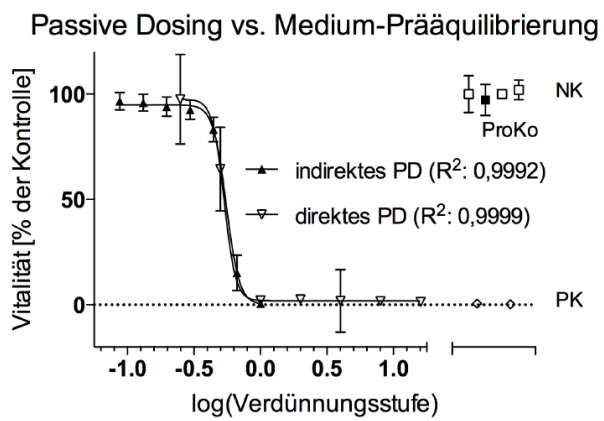


Abbildung 2.8: Schematische Darstellung der drei unterschiedlichen Ansätze zur Exposition – Solvent Spiking, direktes Passive Dosing (PD) über ein als Reservoir fungierendes Polymer und indirektes Passive Dosing über prääquilibriertes Medium.

OASIS HLB pre-loaded with PCP and phenanthrene

Direct passive dosing compared to pre-equilibration of cell medium in Neutral Red cytoxicity bioassay



Both approaches producing comparable results

OASIS HLB can be used as a passive dosing phase

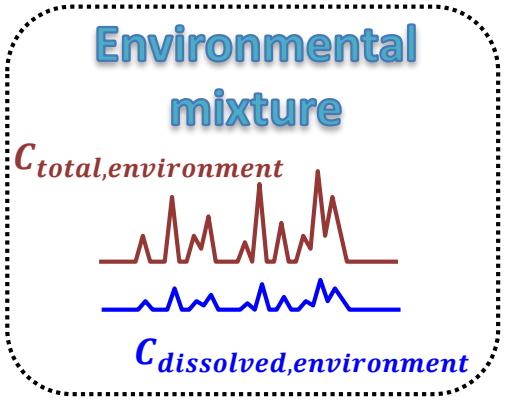
Why not use OASIS HLB directly for equilibrium passive sampling and dosing?

Master thesis: David Kampfer, RWTH Aachen, 2017.

Thank you

Kilian Smith
kilian.smith@bio5.rwth-aachen.de

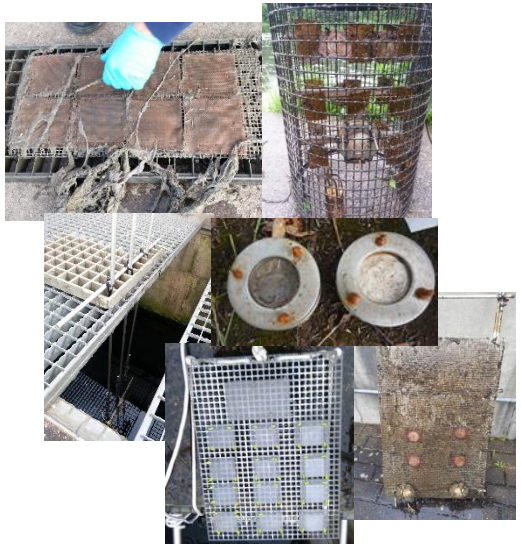
The starting point …..



In the field, biological effects result from exposure to mixtures of organic (and other) contaminants

Organism uptake driven by the bioavailable dissolved concentrations of the mixture constituents

Passive sampling and mixture toxicity



Passive sampling:

- *in situ* sampling in **different matrices** (e.g., water, sediment, soil)
- **complete mixture** profile sampled (even if not completely analyzed)
- **bioavailable dissolved levels** sampled

Measuring environmental mixture toxicity using laboratory bioassays requires:

✓ • Sampling the **complete mixture profile** including unknown compounds

✓ • Sampling the constituents at their **bioavailable levels**

? • **Reproducing and maintaining the bioavailable mixture profile** in the bioassay

Seems to be a niche for polymers with a high affinity for both polar and non-polar compounds .....

Ideal would be a single phase polymer with similar characteristics to silicone

..... but also a high affinity for polar **and** non-polar compounds

Does this exist?