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High Losses and Crossover of Semi-Volatile and Hydrophobic Test Chemicals in *In Vitro* Assays Conducted in 96 Well Plates

Heidi Birch[†], Nynke Kramer[‡] and Philipp Mayer[†]

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Constant and defined exposure in *in vitro* tests can

- increase validity of *in vitro* test results
- increase sensitivity: Left shifting of dose response curves
- increase repeatability
- increase comparability between *in vitro* assays
- facilitate Quantitative *in vitro* to *in vivo* extrapolation (QIVIVE)

Time-Resolved Freely Dissolved Concentrations of Semivolatile and Hydrophobic Test Chemicals in In Vitro Assays—Measuring High Losses and Crossover by Headspace Solid-Phase Microextraction

Heidi Birch,^{*,†} Nynke I. Kramer,[‡] and Philipp Mayer[†]



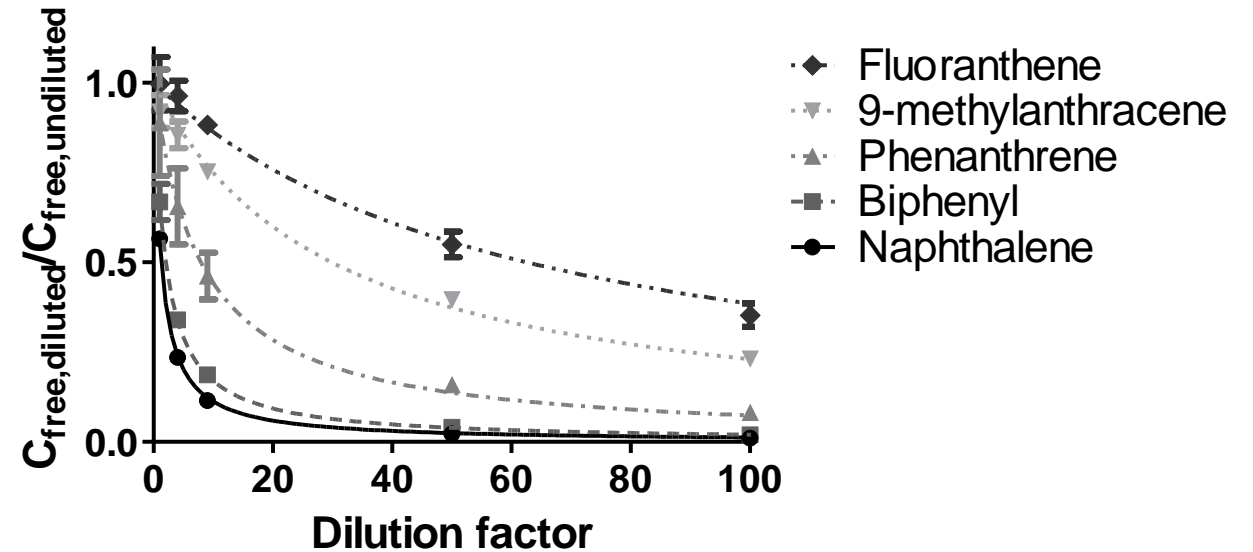
Free fractions – measurements in large volume

- 3 Media spiked with 24 chemicals
- Dilution factors of 1-1000

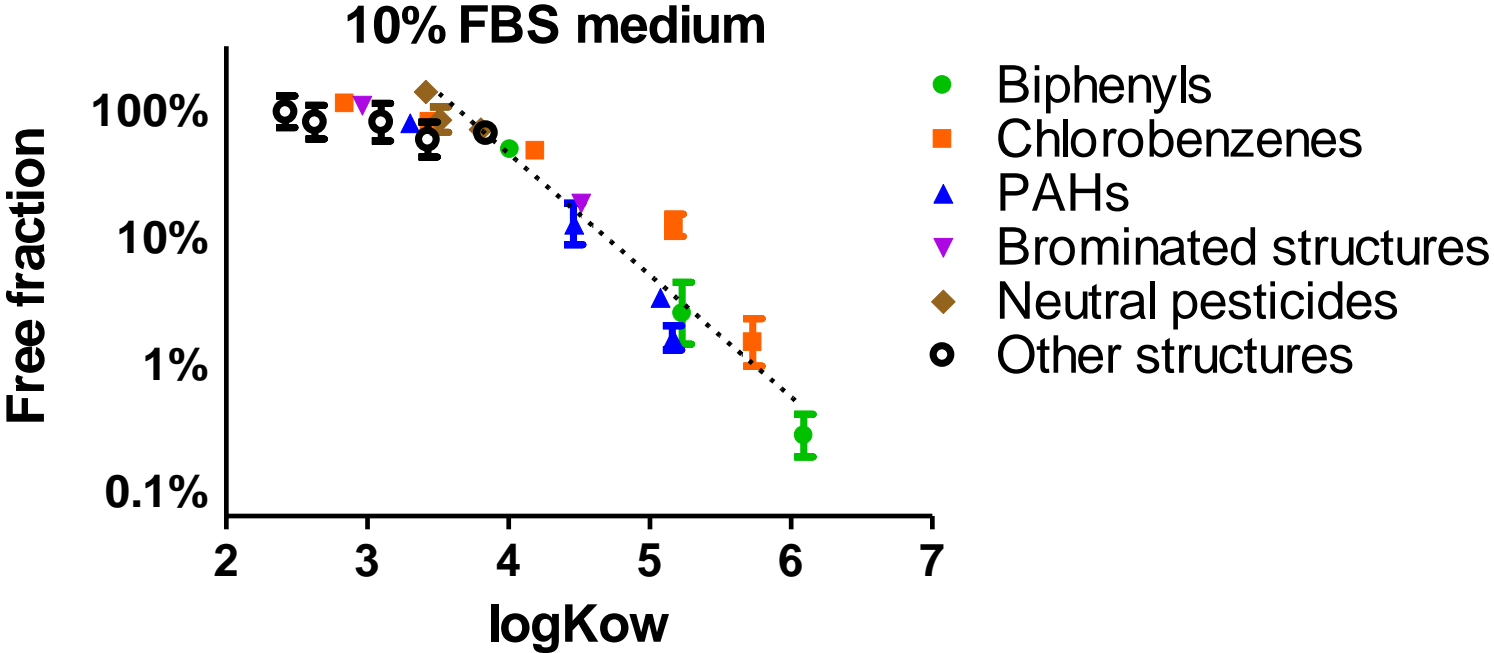


- HS-SPME: C_{air} proportional to C_{free}

$$\frac{C_{free,diluted}}{C_{free,undiluted}} = 1 - \frac{DF}{DF+ff}$$



Free fractions - results



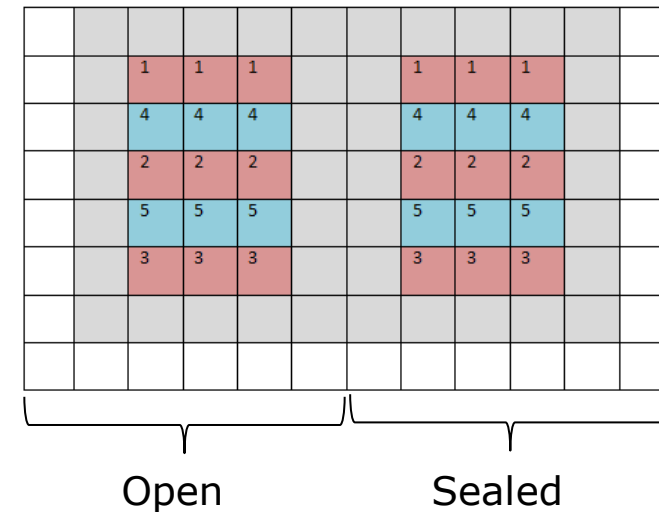
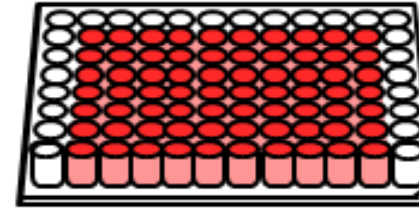
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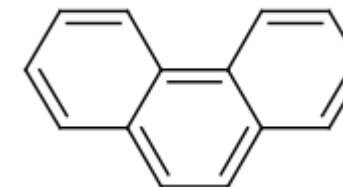


$C_{\text{medium}}(t)$ - test substance losses from wells

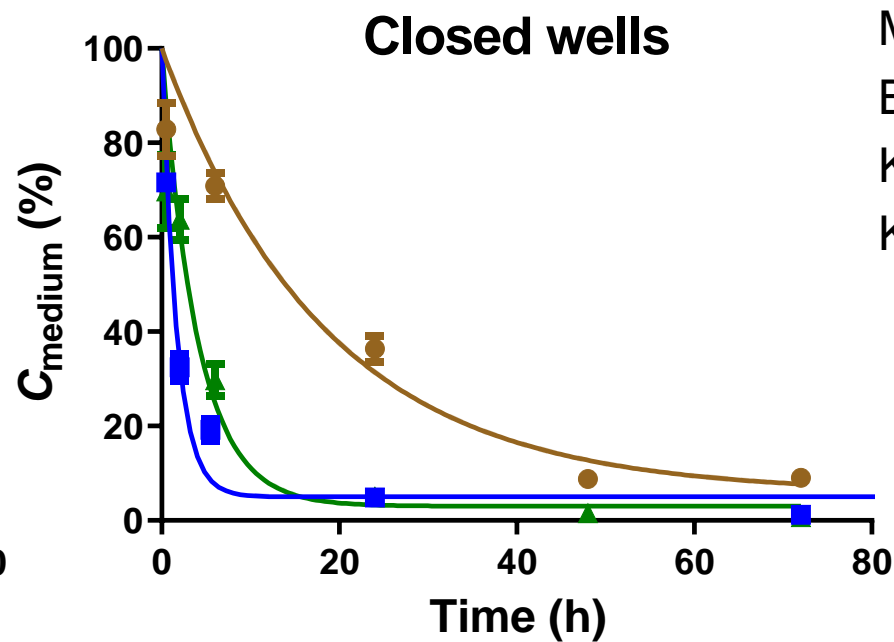
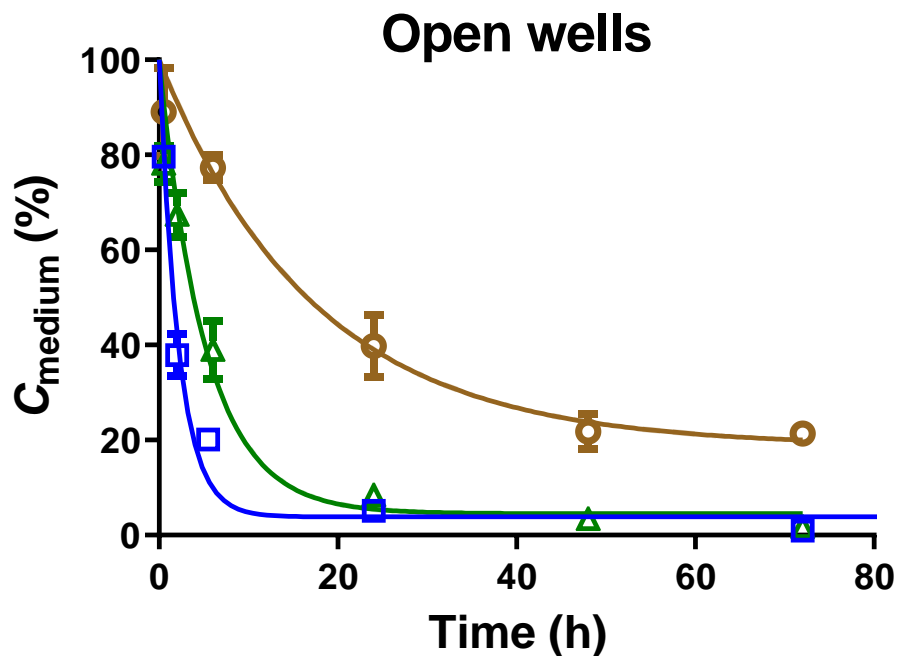
- 96 well microplates, spiked with 24 chemicals
- Incubation at 37°C for 1-72 h
- Entire plates sacrificed for each measurement
- 100 μL withdrawn from each well, 3 samples pooled in 20 mL HS-vial for HS-SPME
- $C_{\text{medium}}(t)$ measured relative to spiked medium
- Cross-over: red=spiked wells, blue=non-spiked
- Half of the plate sealed with aluminum microplate sealing tape



Losses of phenanthrene at 37°C

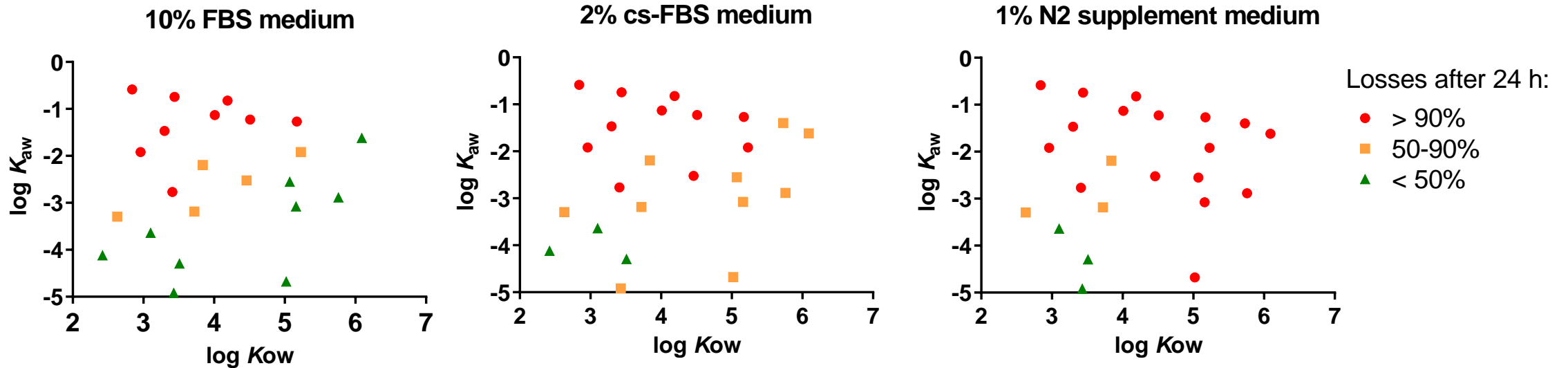


Melting point=101° C
 Boiling point=339° C
 $K_{OW}=4.46$
 $K_{AW}= 0.003$ L/L



■ 1% N₂-supplement medium
 ▲ 2% cs-FBS medium
 ● 10% FBS medium

Evaporative and sorptive losses in open plates



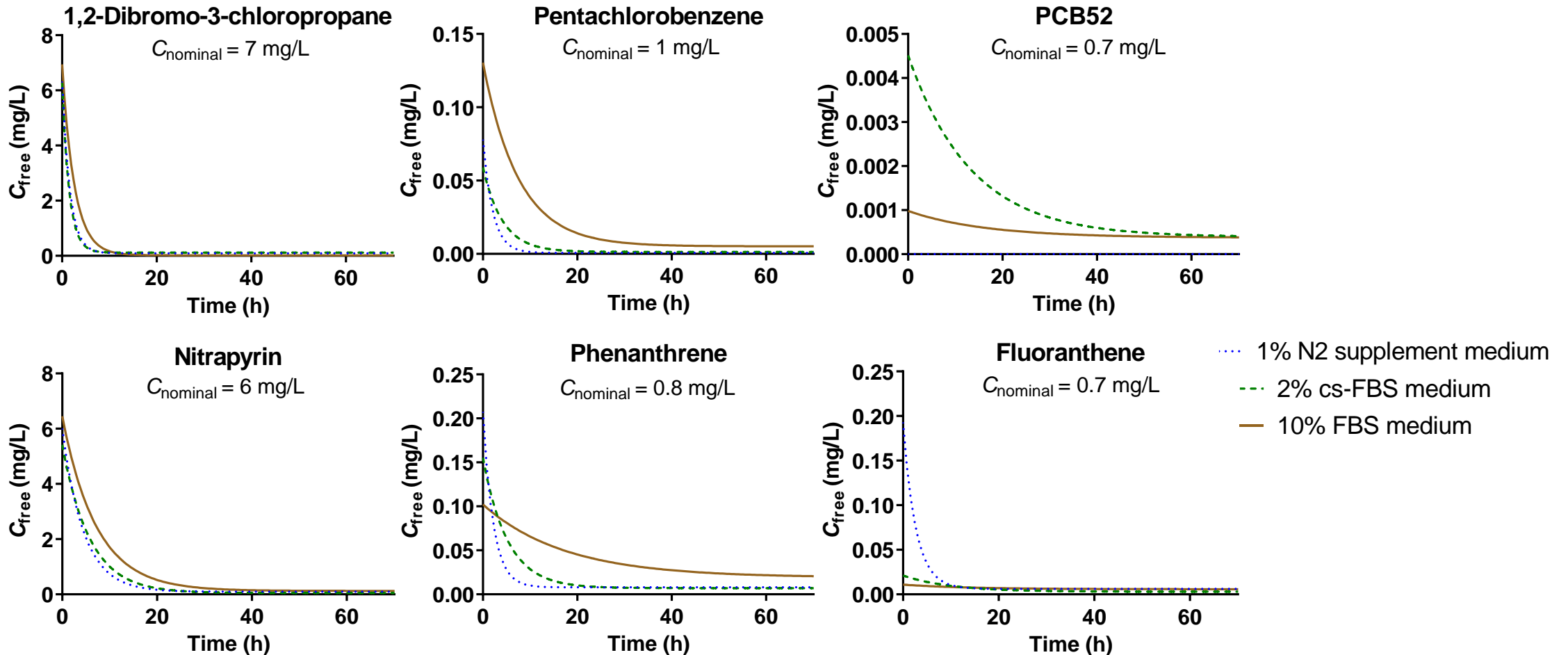
- High volatile and sorptive losses
- Losses were higher than literature reported losses at $\sim 20^\circ\text{C}$
- Higher FBS content reduced losses for high K_{ow} chemicals

Time-Resolved Freely Dissolved Concentrations of Semivolatile and Hydrophobic Test Chemicals in In Vitro Assays—Measuring High Losses and Crossover by Headspace Solid-Phase Microextraction

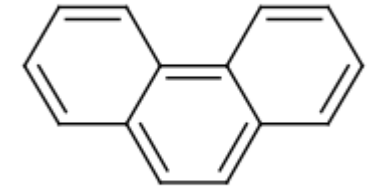
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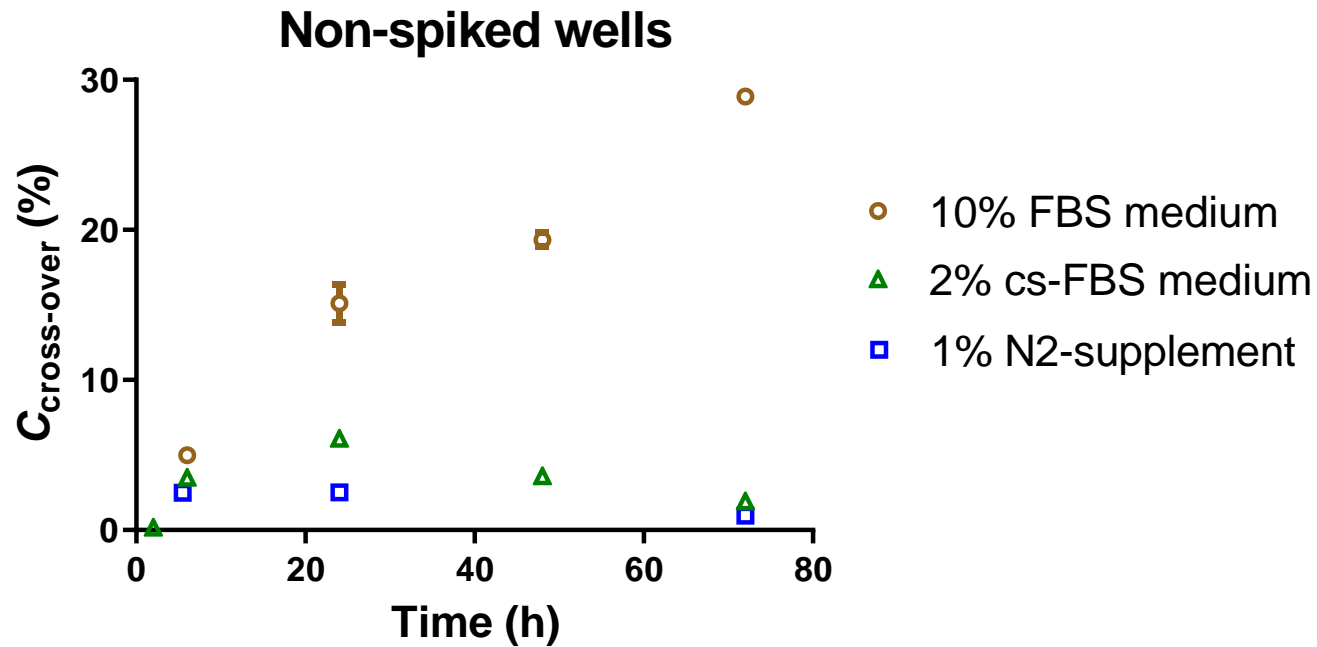
Freely dissolved concentrations



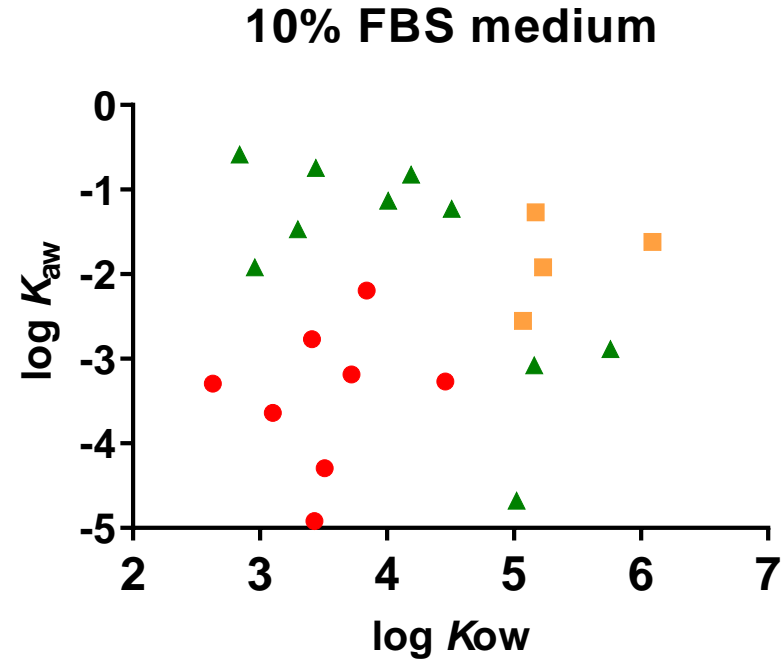
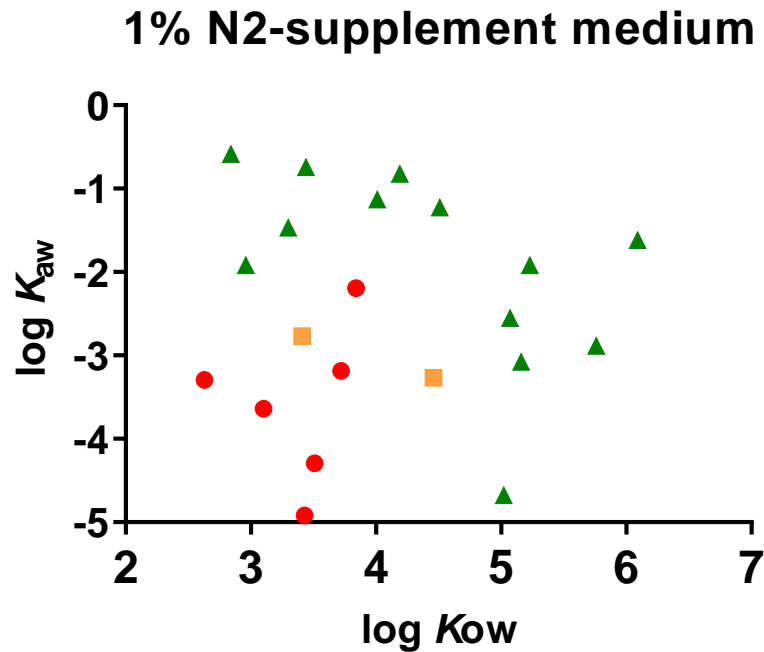
Cross contamination - phenanthrene



Melting point=101° C
 Boiling point=339° C
 $K_{OW}=4.46$
 $K_{AW}= 0.003$ L/L



High cross-over for chemicals with lower losses



Crossover after 24 h:

- >5%
- 2-5%
- ▲ <2%

Conclusions and implications

96 well plates are not suited for semi-volatile and hydrophobic chemicals!

High losses or cross-over within chemical space of (1) solvents, (2) aliphatic and monoaromatic hydrocarbons, (3) 2-4 ringed PAHs (4) phthalates, (5) perfumes and essential oils, (6) cyclic siloxanes, (7) several POPs, (8) some pesticides

There is a need for

1. Test systems that minimize losses
2. Dosing methods to control *in vitro* exposure
3. Analytical confirmation of exposure
4. Exposure confirmation included in good *in vitro* reporting standards
5. Models to assess and predict losses and cross-contamination

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LRI ECO 36 project

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ECETOC Monitoring Team: Todd Gouin (chair), Mark Cronin (LJMU), Burkhard Flick (BASF), Reinhard Kreiling (Clariant), Martijn Rooseboom (Shell)

Thank you for your attention!