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

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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)

Chemical Research in Toxicology

Article

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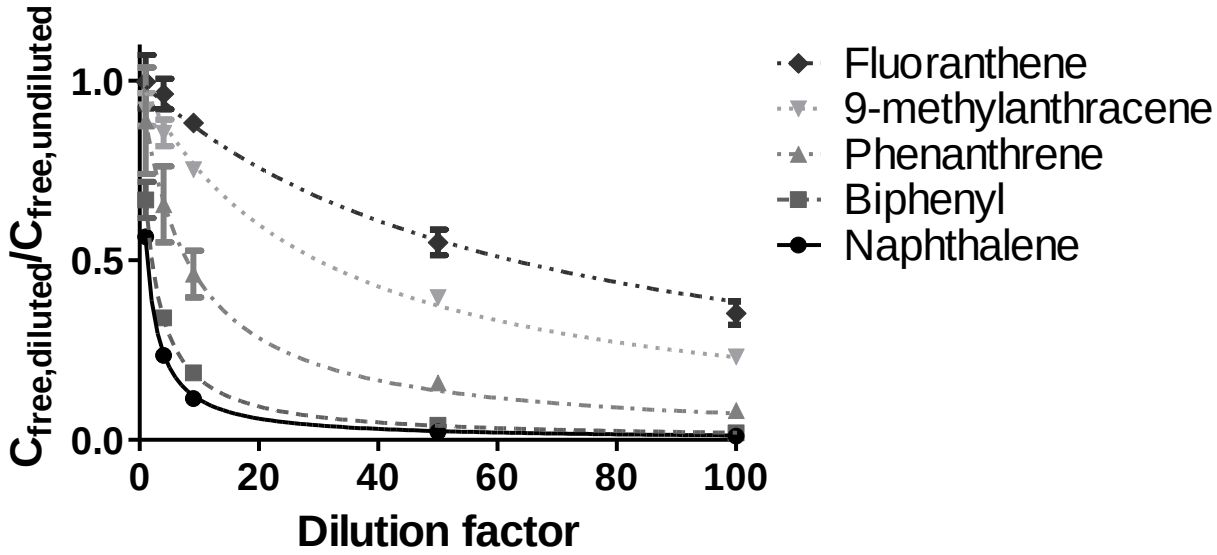
Time-Resolved Freely Dissolved Concentrations of Semivolatile and Hydrophobic Test Chemicals in In Vitro Assays—Measuring High Losses and Crossover by Headspace Solid-Phase Microextraction

Heidi Birch,*,† Nynke I. Kramer,‡ and Philipp Mayer†

Free fractions – measurements in large volume

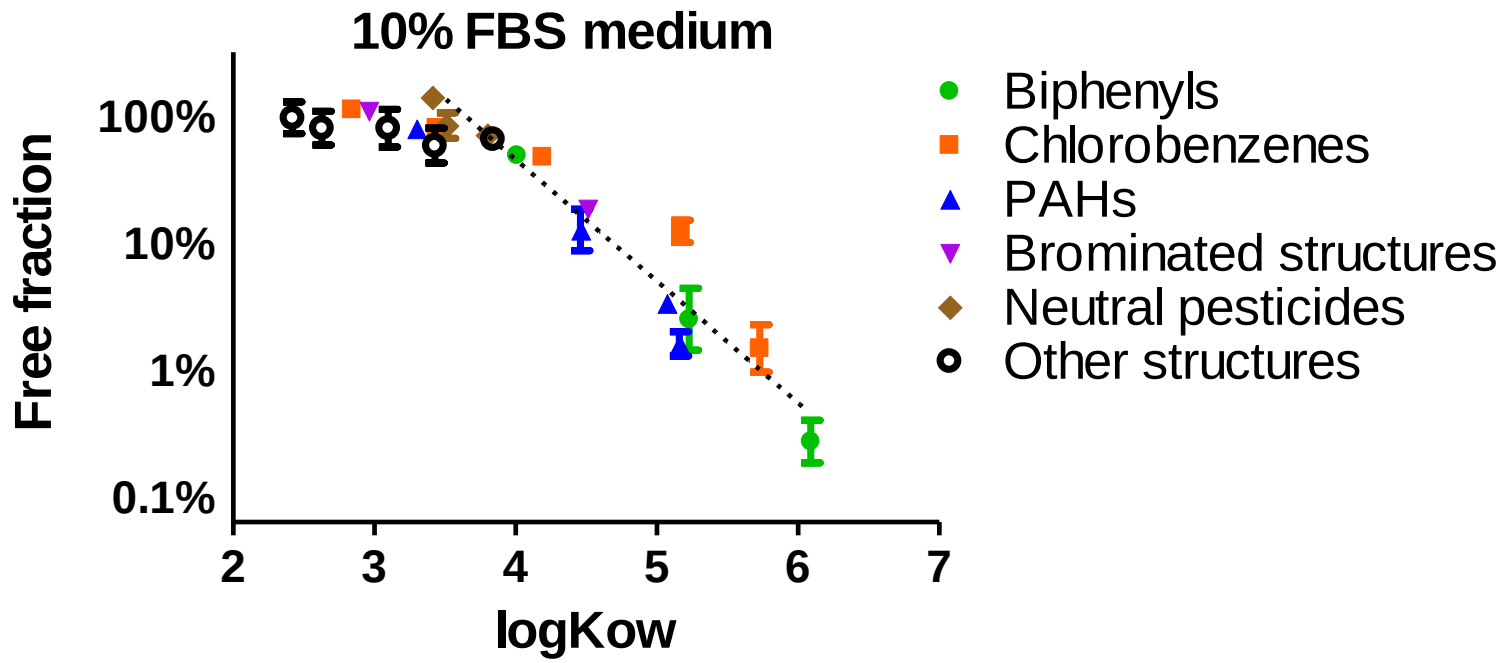
- 3 Media spiked with 24 chemicals
- Dilution factors of 1-1000

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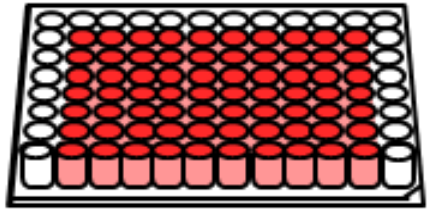
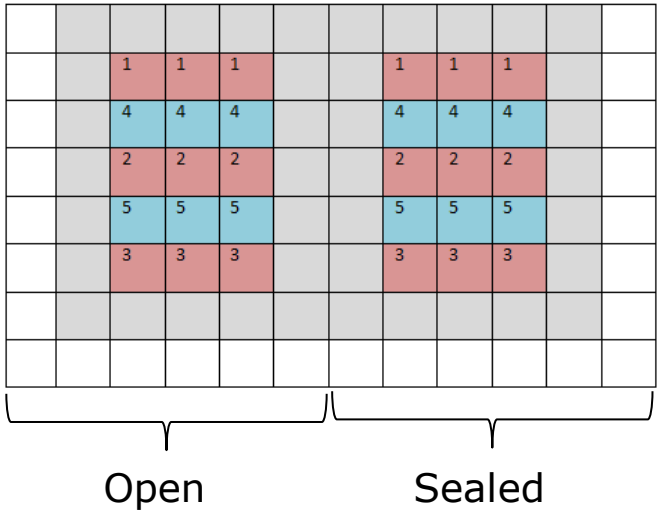
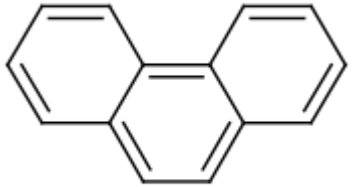
$C_{medium}(t)$ - test substance losses from wells

- 96 well microplates, spiked with 24 chemicals
- Incubation at 37℃ 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
- $C_{medium}(t)$ 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

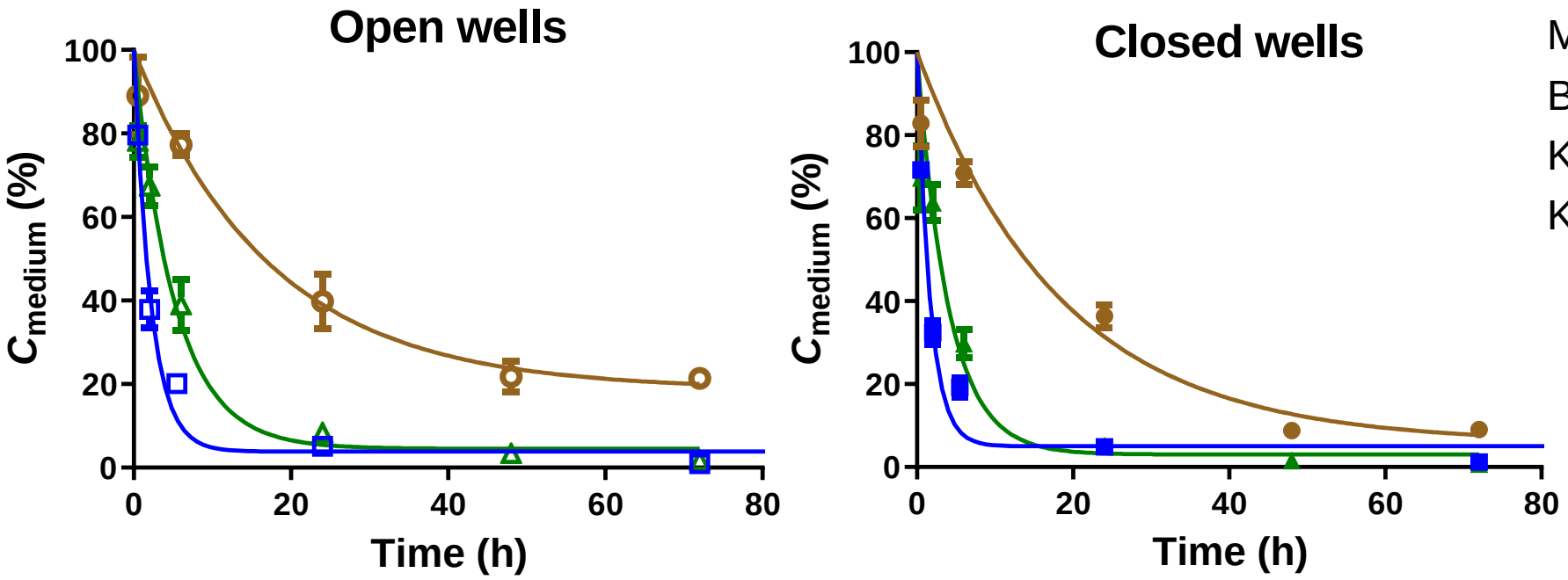


Melting point=101° C

Boiling point=339° C

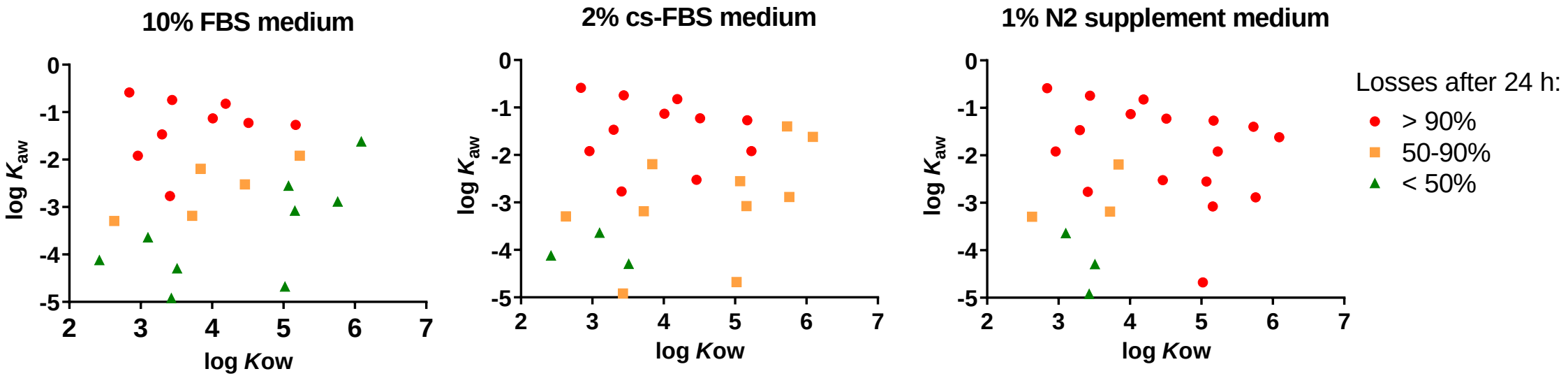
K_{OW}=4.46

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 ~20℃
- Higher FBS content reduced losses for high K_{ow} chemicals

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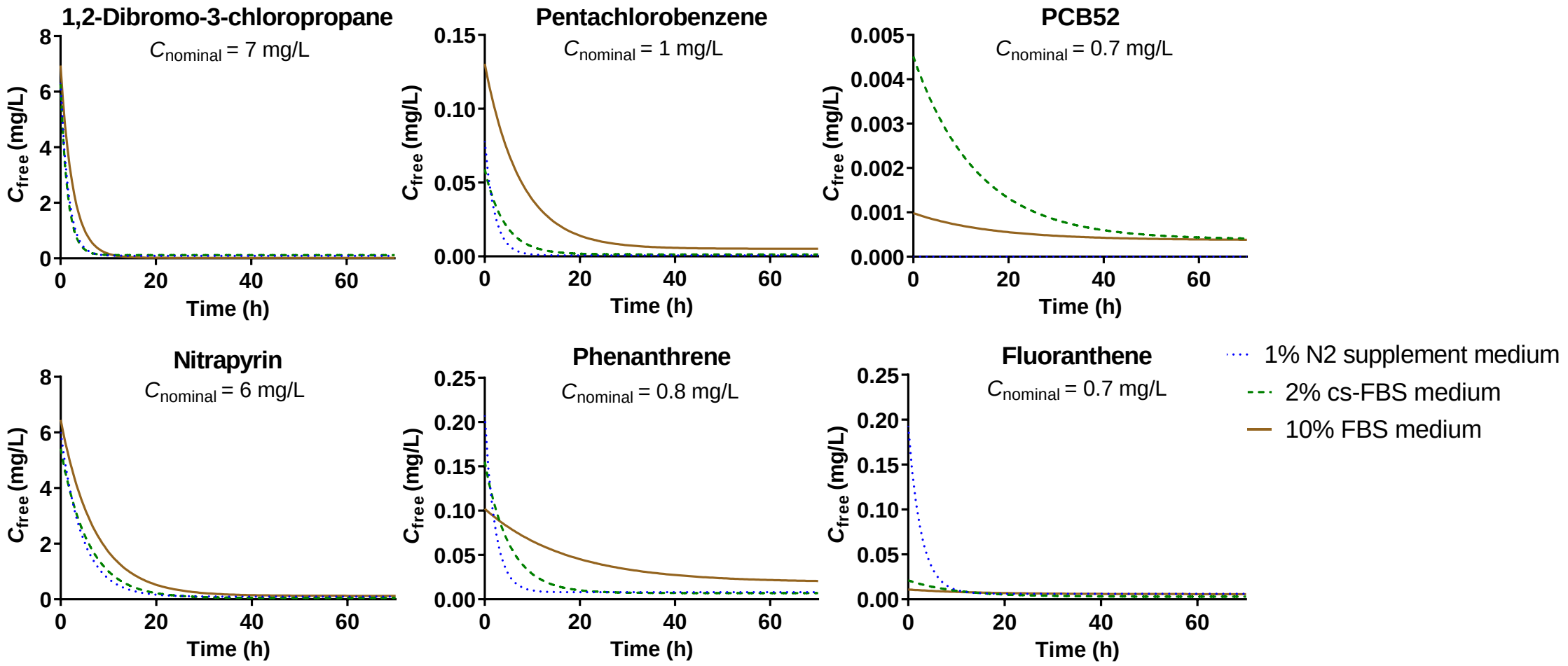
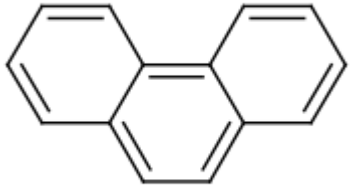
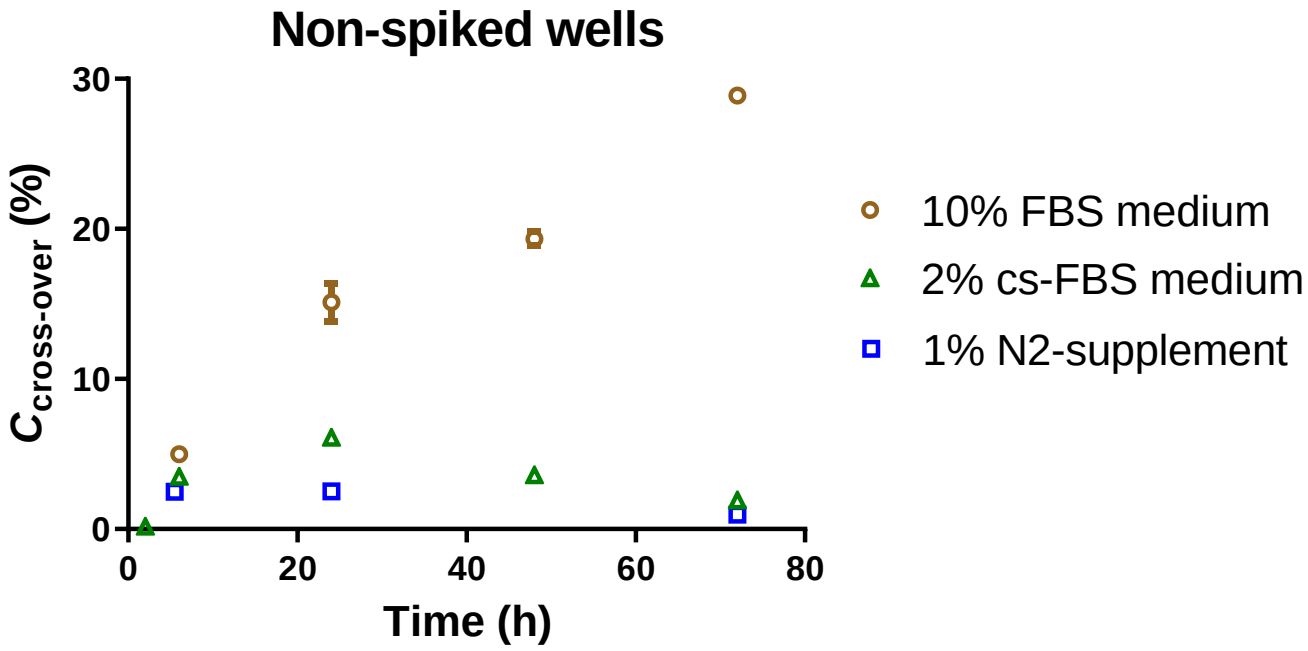
Freely dissolved concentrations



Cross contamination - phenanthrene





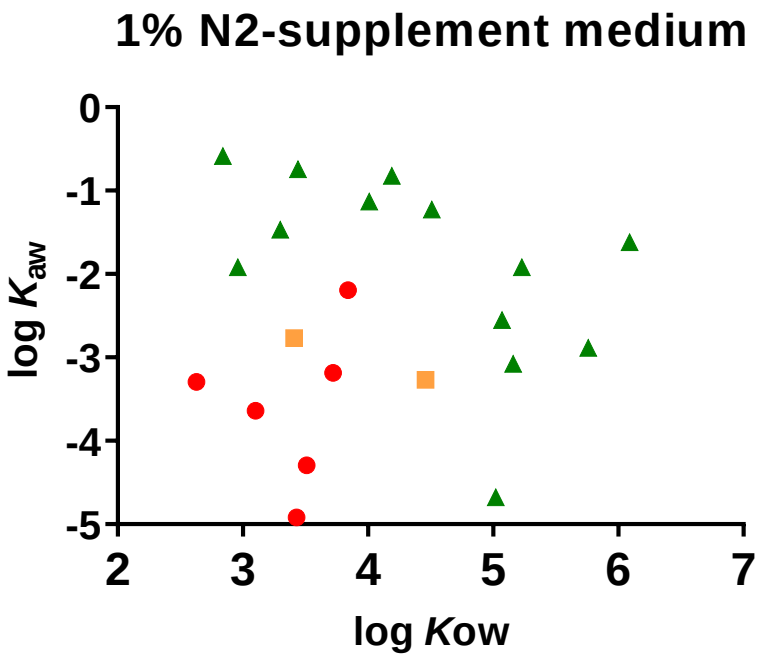
Melting point=101˚ C

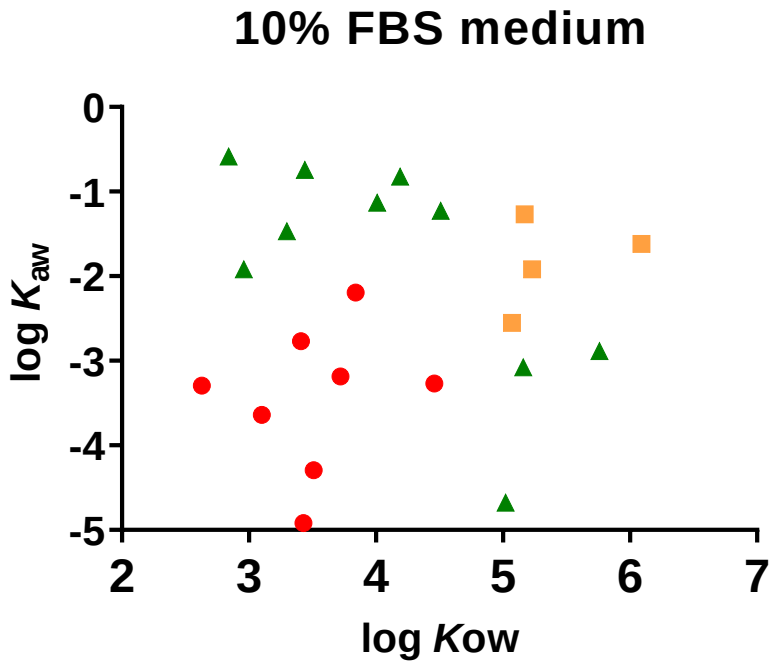
Boiling point=339˚ C

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) parfumes 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 asses and predict losses and cross-contamination

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LRI ECO 36 project

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Thank you for your attention!