University of Applied Sciences Hamburg

HAW Hamburg

Passive dosing of polycyclic aromatic hydrocarbons in the marine algae test using silicone O- rings

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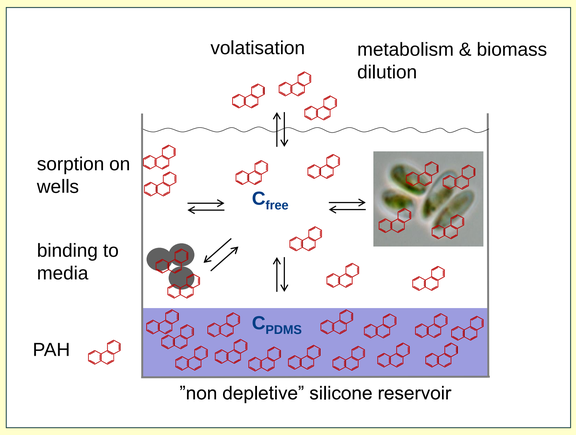
Introduction

Testing hydrophobic organic compounds (HOCs) in aquatic toxicity tests is difficult due to compound losses through volatilization, sorption to the test vessel and culture medium constituents. This results in poorly defined exposure, the bioavailable concentration is reduced and concentration-effect-relation might be underestimated.
Passive dosing can overcome these problems by the continual partitioning of HOCs from a dominating reservoir loaded in a biologically inert polymer such as silicone (1-4). This procedure provides defined and constant freely dissolved concentrations and eliminates spiking with cosolvents.

Passive dosing principle



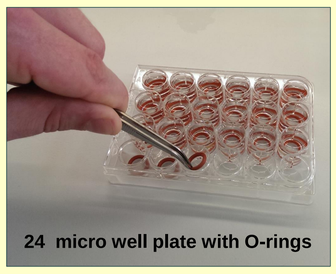
Advantages Passive Dosing

(1) Control of C_{Free} and not C_{total}
(2) Constant C_{free} during the whole test (72h)
(3) No solvents or cosolvens

Passive Dosing Material: silicone (PDMS)

(1) Chemically inert and biocompatible
(2) High PAH capacity (no depletion)
(3) Linear PAH partitioning over full concentration test range

Material & Methods Passive dosing vs. Standard dosing


24 micro well plate with O-rings

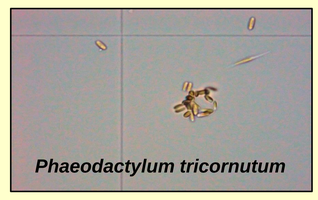
	Passive dosing	Standard dosing
Method	(1) Pre-cleaning the O-rings (2) Loading silicone with PAHs to required level from methanol solution (saturation and serial dilutions) (3) Cleaning of the O-rings with a small volume of water (4) Equilibration of the loaded O-ring with the test medium	(1) Direct Serial dilution of selected PAHs in ASW water (2) Dilution from ASW solubility and dilution series (1:1 to 1:32)
Exposure control	Controlled by equilibrium partitioning between loaded PDMS and test medium	not possible
Exposure confirmation	analysis of PAHs in silicone	not possible

Marine algae test with *Phaeodactylum tricornutum*

Criteria for the selection of the marine algae toxicity test

- Marine toxicity data are underrepresented
- International standardized test procedure (ISO EN DIN 10253, 2006)
- Important parameter (reproduction - growth rate (E_rC_{50}) in 72 h
- Marine diatom *Phaeodactylum tricornutum* shows three morphological forms – pelagic and benthic - of highly interest with respect to the bioavailability of HOCs

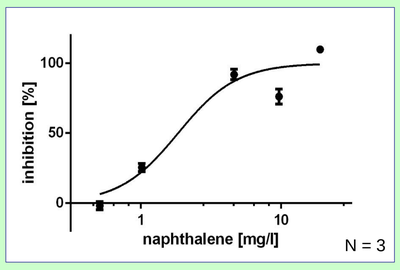
- All tests were conducted in 24 micro well plate
- artificial seawater (ASW) was used
- All validity criteria were fulfilled
- EC_{50}-values were calculated with GraphPad Prism 6.0


Phaeodactylum tricornutum

Results

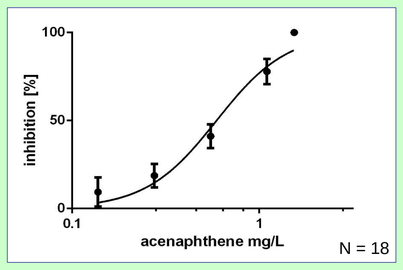
passive dosing

naphthalene



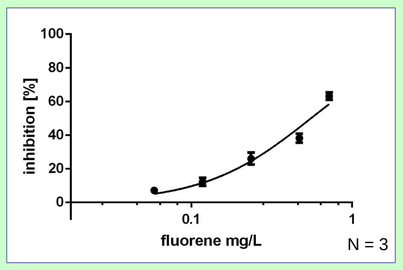
Passive dosing naphthalene (Naph)
E_rC_{50} = 1.84 mg/l (95% CI 1.289 to 2.638)

acenaphtene



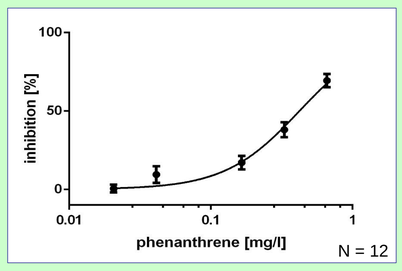
Passive dosing acenaphthene (Ace)
E_rC_{50} = 0.59 mg/l (95% CI 0.56 to 0.62)

fluorene



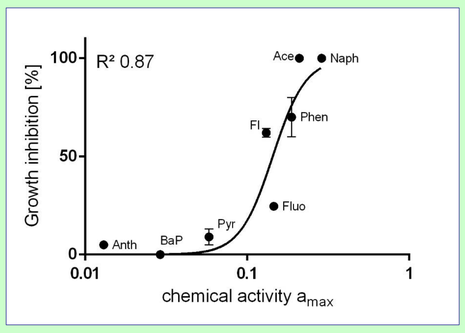
Passive dosing fluorene (Fl)
E_rC_{50} = 0.55 mg/l (95% CI 0.49 to 0.62)

phenanthrene

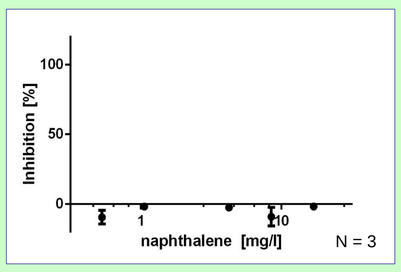


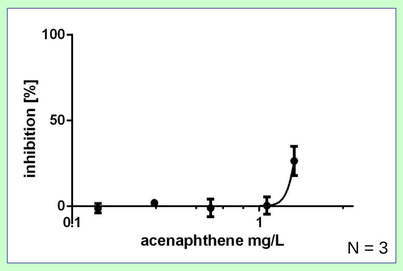
Passive dosing phenanthrene (Phen)
E_rC_{50} = 0.42 mg/l (95% CI 0.37 to 0.47)

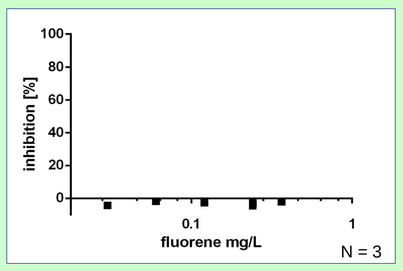
Chemical activity

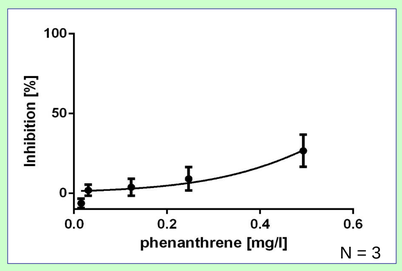


standard dosing





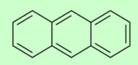




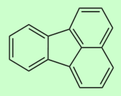
Standard dosing phenanthrene
E_rC_{50} = 0.87 mg/l (95% CI 0.51 to 1.49)

Growth rate inhibition in passive dosing tests at saturation < 25% for:

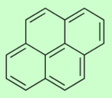
anthracene (Anth)



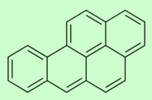
fluoranthene (Fluo)



pyrene (Pyr)



benzo(a)pyrene (BaP)



Discussion

(1) Comparison of the E_rC_{50} values passive dosing vs. standard dosing:
→ underestimation of the effects or no effects when using nominal standard dosing
→ probably reasons: sorptive losses and limiting dissolution kinetics

(2) Passive dosing concentration-response curves were more reproducible

(3) Curves shifted towards lower concentrations by several orders of magnitude

(4) One activity-response model could be fitted to all data, which yielded an effective chemical activity (Ea_{50}) of 0.14, which is slightly higher compared to recently published studies with *Daphnia magna* (Ea_{50}=0.036) and *Danio rerio* embryos (Ea_{50}=0.059-0.089)

Conclusions

(1) Response is clearly not only dependent on the potency of the compounds, but also on its supply, sorption and consumption during the assay.

(2) Passive dosing is a practical and economical way of improving the exposure of HOCs in
→ aquatic toxicity tests
→ bioconcentration tests

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(2) Smith K.E.C., Oostingh G.J., Mayer P. (2010) Chem. Res. Toxicol. 23:55-65
(3) Rojo-Nieto E, Smith KEC, Perales-Vargas-Machuca JA and P Mayer (2012) Aquatic Toxic. 120-121:27-34
(4) Seiler TB, Best N, Fernqvist MM, Hercht H, Smith KEC, Braunbeck T, Mayer P and H Hollert (2014) Chemorsphere 112:77-84

Acknowledgements:
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FONA Forschung für nachhaltige Entwicklungen BMBF

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