Passive dosing and aquatic toxicity testing of complex organic mixtures
Complex organic mixtures

- UVCBs: Unknown or Variable composition, Complex reaction products or Biological materials
- Hydrophobic and volatile
- Petroleum products and essential oils

Photo: Marco Verch
Photo: Chris Gin
Photo: www.healthline.com
Characteristics

• Numerous chemicals with different physicochemical properties
• The composition partly unknown
• The variability of composition is relatively large or poorly predictable

Challenges:

• Difficult to establish and maintain exposure concentration in aquatic toxicity tests
• Difficult to define the exposure levels and the mixture composition
• Difficult to assess the toxicity of the mixture as a whole
Baseline versus Excess toxicity

Baseline toxicity
• minimum toxicity due to distortion of membrane function
• reversible partitioning into lipid membrane to a critical level (40-160 mM) [1]

Excess toxicity
• higher toxicity due to other modes of action

For mixtures
• Baseline toxicity expected at sum concentration in membranes of 40-160 mM
• Excess toxicity indicated when effects are exerted below this range

Aims

• Develop passive dosing methods for dose-response testing of UVCB mixtures
• Determine the toxicity of petroleum products and essential oils to *Daphnia magna*
• Link toxicity to sum concentrations in donors and membranes
• Baseline or excess toxicity?
Test mixtures and organism

Petroleum products
- Multi-parameter Certified Reference Material – Diesel (CRM-MPGO)
- Certified Reference Material – Kerosine (CRMU-DIKR)
- Cracked Gas Oils

Essential oils
- Fir oil Siberia
- Cedarwood oil USA Virginia type Opur
- Lavender oil Barreme type

Toxicity test
- 48 hour acute Daphnia magna immobilization test (OECD 202)
1) Silicone Passive Dosing  
(Dual medium, non-depletion & equilibrium)

Passive dosing donor:
• PDMS silicone rods of 0.5 g
• Positioned in aqueous medium & headspace

Donor loading:
• direct immersion in excess UVCB (saturation)
• UVCB in silicone (w/w, %) (dose-response)
• rolling for 48 h
2) Headspace Passive Dosing (HS-PD)
(Headspace only, non-depletion & equilibrium)

Passive dosing donor:
• Pure triglyceride of fractionated plant fatty acids (Miglyol oil 812)

Donor solutions:
• Pure test mixture (saturation) [2]
• UVCB in Miglyol oil (w/w, %) (dose-response) [3]

Headspace GC-MS

Exposure at saturation
Headspace concentrations at saturation in 2 passive dosing systems referenced against pure UVCBs

Concentration & composition of Fir oil
Chemical activity of components in dilution series

\[
\frac{a_{\text{component}}}{a_{\text{saturation}}} = \frac{\text{peak area}_{\text{component}}}{\text{peak area}_{\text{saturation}}}
\]
Exposure confirmation at saturation

CRMU-Kerosine

HS-PD
Silicone PD
Reference (saturated vapor)
Non-linear partitioning at constant composition

![Graph showing non-linear partitioning at constant composition](image)
1) Daphnia immobilization – Silicone PD

- In the left graph, the x-axis represents the concentration of silicone (mass %) and the y-axis shows the immobility (%) of Daphnia. The graph compares the effects of silicone on the immobilization of Daphnia from different essential oils: Fir, Cedarwood, and Lavender.

- The right graph similarly plots the immobility (%) against the concentration of silicone (mass %), but it includes data on Diesel, Kerosene, and Cracked gas oil.

- The bottom graphs show the concentration of the membrane = donor (mmol kg⁻¹) on the x-axis and the immobility (%) on the y-axis, comparing the effects of silicone on Daphnia immobilization with different oil types.
2) Daphnia immobilization – HS-PD

![Graphs showing immobilization of Daphnia vs. lipid concentration and membrane donor concentration.]

- **Fir**, **Cedar wood**, and **Lavender**
- **Diesel**, **Kerosene**, and **Cracked gas oil**

**Legend:**
- Blue circle: Fir
- Red square: Cedar wood
- Purple triangle: Lavender
- Red circle: Diesel
- Green square: Kerosene
- Blue triangle: Cracked gas oil
Conclusions

• Exposure at saturation confirmed in the two PD methods by headspace GC-MS using saturated vapor as thermodynamic reference.

• Toxicity results consistent between dosing methods and consistent with baseline toxicity. Lack of excess toxicity specific to test organism, duration and endpoint.

• Headspace PD avoids biofouling & micro-droplets, but limited to (semi)volatiles.

• Silicone PD extends to less volatile constituents, but micro-droplet can be formed near saturation level.

• Passive dosing provided an improved experimental basis to assess toxicity of complex organic mixtures of volatile hydrophobic chemicals.
We’d like to thank

Laboratory assistance

Susanne Kruse
Hanne Bøggild

Test mixture supply

Funding

Thank you for your attention!
Headspace Passive Dosing of Volatile Hydrophobic Organic Chemicals from a Lipid Donor—Linking Their Toxicity to Well-Defined Exposure for an Improved Risk Assessment

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Supporting Information

ABSTRACT: High hydrophobicity and volatility of chemicals often lead to substantial experimental challenges but were here utilized in headspace passive dosing (HS-PD) to establish and maintain exposure: the pure chemical served as a passive dosing donor for controlling exposure at saturation, whereas triglyceride oil containing the chemical was used to control lower exposure levels. These donor solutions were added to glass inserts placed in the closed test systems. Mass balance calculations confirmed a dominant donor capacity for all chemicals except isooctane. This HS-PD method was applied to algal growth inhibition and springtail lethality tests with terpenes, alkanes, and cyclic siloxanes. Headspace concentrations above the lipid donors were measured for three chemicals to determine their chemical activity, using saturated vapor as the analytical standard and thermodynamic reference. Toxicity was related to chemical activity and calculated concentrations in membranes at equilibrium with the lipid donor. For both tests and all chemicals, toxic effects were observed within or above the reported range for baseline toxicity, meaning that no excess toxicity was observed. The toxicity of siloxanes was markedly higher to the terrestrial springtail than the aquatic algae, which is consistent with a more efficient mass transfer of these volatile hydrophobic chemicals in air compared to water.
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<th>CAS No.</th>
<th>Without PD Air</th>
<th>Water</th>
<th>Lipid-based PD Lipid</th>
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<th>Silicone-based PD Silicone</th>
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