UVCB fate-directed toxicity testing and risk assessment

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1. UVCB-FATETOX (CEFIC LRI ECO 42)
2. Test Exposure (losses, passive dosing & testing)
ECO 42 outline

Focus on petroleum & essential oil UVCBs
How to obtain defined & constant exposure in tests and research?

Nominal concentrations ≠ exposure concentrations

Strategies to overcome:
• Avoid or minimize losses: Non-depletion testing
• Model the effective exposure
• Measure the effective exposure
• Control the effective exposure: **Passive dosing**
Testing mixture: losses alter exposure levels & composition

Mixture composition

Exposure level

Time

3 compounds
Evaporative and sorptive loss – composed mixture in 96 well plates
CEFIC ECO 36 project - Heidi Birch (in revision)

\[ C_{\text{free}}(t) = \text{free fraction} \times C_{\text{medium}}(t) \]

Diagram:
- Graph showing time (h) vs. mg/L.
- Tubes indicating medium dilution method.
- Grid of wells demonstrating measured losses.
Evaporative and sorptive losses – composed mixture in 96 well plates
CEFIC ECO 36 project - Heidi Birch (in revision)

- High volatile and sorptive losses
- Losses increased with $K_{aw}$ and $K_{ow}$
- Higher losses at 37°C than ~20°C (literature)
Accelerated passive dosing of hydrophobic volatile UVCBs
PhD study Rikke Hammershøj (main funding by Concawe)

1. Passive dosing donor with high A and A/V
2. Loading of UVCB by direct addition or immersion
3. Add water and roll for passive dosing
Fast Dosing Kinetics

Headspace-SPME measurements of dosed water

![Graph showing equilibration time and passive dosing peak height for different substances.]
Vary UVCCB concentration at constant composition

For application to biodegradation testing of UVCCBs see our poster:

Rikke Hammershøj: “Investigating the concentration effect on biodegradation kinetics of two hydrophobic UVCCB substances”. See poster.
Determining Biodegradation Kinetics of Hydrocarbons at Low Concentrations: Covering 5 and 9 Orders of Magnitude of $K_{ow}$ and $K_{aw}$

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Supporting Information

**ABSTRACT**: A partitioning-based experimental platform was developed and applied to determine primary biodegradation kinetics of 53 hydrocarbons at ng/L to μg/L concentrations covering C8–C20, 11 structural classes, and several orders of magnitude in hydrophobicity and volatility: (1) Passive dosing from a loaded silicone donor was used to set the concentration of each hydrocarbon in mixture stock solutions; (2) these solutions were combined with environmental water samples in gastight auto sampler vials for 1–100 days incubation, and (3) automated solid phase microextraction (SPME) coupled to GC-MS was applied directly on these test systems for measuring primary biodegradation relative to abiotic controls. First order biodegradation kinetics were obtained for 40 hydrocarbons in activated sludge filtrate, 18 in seawater, and 21 in lake water. Water phase half-lives in seawater and lake water were poorly related to hydrophobicity and volatility but were, with a few exceptions, within a factor of 10 or shorter than BioHCwin predictions. The most persistent hydrocarbons, 1,1,4,4,6-pentamethyldedecalin, perhydropyrene, 1,2,3,6,7,8-hexahydropyrene, and 2,2,4,4,6,8,8-heptamethylnonane, showed limited or inconsistent degradation in all three environmental media. This biodegradation approach can cover a large chemical space at low substrate concentrations, which makes it highly suited for optimizing predictive models for environmental biodegradation.
Mixture Effects on Biodegradation Kinetics of Hydrocarbons in Surface Water: Increasing Concentrations Inhibited Degradation whereas Multiple Substrates Did Not

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Supporting Information

ABSTRACT: Most biodegradation tests are conducted using single chemicals at high concentrations, although these chemicals are present in the environment as mixtures at low concentrations. A partitioning-based platform was recently developed for biodegradation testing of composed mixtures of hydrophobic chemicals at ng/L to μg/L concentrations. We used this platform to study the concentration and mixture effect on biodegradation kinetics. Biodegradation tests were conducted in 20 mL vials using environmental water samples as inocula. Passive dosing was applied (1) to vary initial test concentrations of individual test compounds and (2) to vary the number of mixture components between 1 and 16. Automated solid-phase microextraction coupled to gas chromatography–mass spectrometry was used to measure substrate depletion relative to abiotic controls. The number of mixture components had no or only a limited effect on the biodegradation half times for three compounds when tested at environmentally relevant concentrations. In contrast, longer lag phases and half lives were observed for single compounds when tested at higher concentrations that approached aqueous solubility. The obtained results support that simultaneous testing of multiple chemicals at low concentrations can accelerate the generation of biodegradation kinetic data, which are more environmentally relevant compared with data from tests conducted with single chemicals at much higher concentrations.
Characterization of hydrocarbons in test material and in extracts of passive dosing

- Hydrocarbon speciation (GCxGC) of CRM-diesel test material and water from passive dosing system
- Passive dosing: Differences in water solubility (aliphatics vs. aromatics) driving the hydrocarbons patterns in water
WAF and Passive Dosing yield similar composition, whereas spiking amplifies the more hydrophobic constituents.
Passive dosing & toxicity testing of UVCBs
PhD study Lam Ngoc Trac

Silicone Passive Dosing

Headspace Passive Dosing
Exposure confirmation at saturation - Headspace GC-MS

CRM-Diesel

Headspace-PD
Silicone PD
Reference: saturated vapor
1. Storage lipid as donor: Right shifting of dose-response curve
2. No sign of excess toxicity for any of the tested UVCBs
Bioaccumulation testing of UVCB mixtures

\[
BCF = \frac{C_{fish}}{C_{water}} = \frac{k_1}{k_T}
\]

- Using single dietary exposure and internal benchmarking to determine the BCF of UVCBs

\(^1\text{Arnot & Gobas 2004}\)
Benchmarking: a way to reduce inter-individual variability

- Correction for growth-dilution
- Correction for differences in feeding behaviour
- Measurements of multiple constituents in one in-vivo experiment

For more details please join our talk:

**Roxana Samson:** “Determining BCFs of essential oil constituents using in-vivo benchmarked dietary exposure studies”. See poster.
We’d like to thank

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...you for your attention!