Investigating closed test setups and application approaches for biodegradation testing of a volatile and hydrophobic substance in surface water (OECD 309)

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Introduction

- Volatile and hydrophobic substances (VHS) are known to present issues in biodegradation testing.
- The OECD 309 test guideline (biodegradation in surface water) faces particular challanges with VHS due to the test matrix and specifies a Henry's law constant (HLC) of 100 Pa m^3 mol^{-1} as an upper limit for test substance volatility.[1]
- Decahydronaphthalene ("decalin", $C_{10}H_{18}$, CAS no: 91-17-8) is a VHS (log K_{OW}: 4.2; HLC: 10,740 Pa m^3 mol^{-1}).
- A previous OECD 309 test conducted with ^{14}C labelled decalin used silicon oil as an application solvent.[2,3] Due to the insoluble properties of silicon oil, no mass balance was determined, and degradation was assessed based on $^{14}CO_2$ formation only.
- The use of silicon oil is expected to impact the partitioning and bioavailability – and hence degradation rate – of decalin under OECD 309 conditions.
- The study aims to investigate elements of the previous OECD 309 test on decalin and explore possible refinements and alternative approaches to achieving a robust OECD 309 test: These investigations were made under three different work packages (WP).

WP 1: Separation and analysis of radioactivity in silicon oil-water

- 80 µL silicone oil spiked with ^{14}C labelled decalin was added to 80 mL water and shaken (130 rpm) for 0.5 hours before allowing to settle.
- Silicone oil (droplets) were separated at first using physical separation using glass pipette. Acetonitrile (50 mL) was added to the water to dissolve/extract the residual silicone oil dispersion.

Results

Table 1. Distribution of applied radioactivity (% AR) in the silicone oil and aqueous phase with silicone oil dispersion which was quantified by extracting with solvent.

Sample	AR (%) Silicone oil droplet	AR (%) water phase + Acetontrile	AR (%) Total recovery
1	45.79	26.47	72.26
2	65.42	13.53	78.95
Mean	55.61	20.00	75.60

- Indirect method (sequential addition of acetonitrile without silicone oil separation) also possible and produced similar recoveries (data not shown).

WP 2: Preparing aqueous application solution using passive dosing

- 2 mm diameter silicon rods (2 x 0.5 g) were each loaded with 500 kBq (~37 µg) ^{14}C labelled decalin dissolved in 2 mL ethanol in closed glass vials.
- Loaded rods were equilibrated with 300 mL water in sealed flasks and sampled over a period of 12 days (0, 1, 3, 7 , 24 ,48, 96, 192, 288 h).
- Remaining radioactivity was extracted from silicone rods using 4 X 10mL acetonitrile.

Results

- Steady state was reached after 96 h. Concentration in the water phase (based on specific radioactivity): 0.87 - 1.07 µg/L (~12 Bq/mL).
- Amount in the water phase was < 1% radioactivity loaded in silicon rods.
- Amount of radioactivity recovered by extraction of rods: 56.5 – 75.7 % AR.
- Amount of radioactivity in the application solutions is not enough to make robust and reliable radioactivity measurement for OECD 309 test.

WP 3: Performance of volatile traps and feasibility of obtaining a mass balance using closed setups

- Six different closed setups were used with different volatile trapping approaches.
- ^{14}C labelled Decalin was prepared in ethanol stock solution and was applied to 80 mL water (concentration: ~50 µg/L) and setups were immediately closed.
- Incubated under shaking (130 rpm) at 12 °C for 14 days before sampling.
- At sampling, headspace was stripped through volatile traps before LSC analysis.

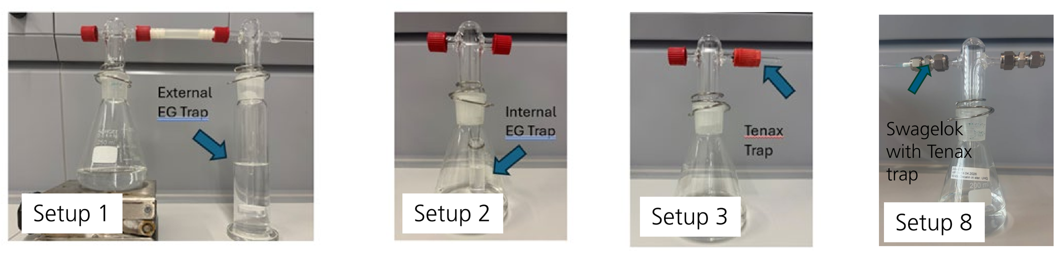

Figure 1. Test setups used in mass balance experiments with different volatile traps.

Results

Table 2. Distribution of applied radioactivity (% AR) in different compartments in the closed setups

Setup	Volatile trap	Trap attachment	Setup closed using	%AR Septum extract	%AR Setup rinsing	%AR Water Phase	%AR Volatile trap	% AR Recovery
1	EG	On sampling	[d]PTFE lined silicone septum	N.D.	N.D.	9.32	3.37	12.69
2	EG	Internal trap	[d]PTFE lined silicone septum	N.D.	N.D.	6.71	6.55	13.25
3	Tenax	On sampling	[d]PTFE lined silicone septum	N.D.	N.D.	8.14	2.24	10.38
4(i)[a]	Tenax	Permanent	[d]PTFE lined silicone septum	N.D.	N.D.	5.46	52.88	58.35
5[b]	Tenax	Permanent	[d]PTFE lined silicone septum	N.D.	N.D.	8.34	53.43	61.78
4(ii)[a]	Tenax	Permanent	[d]PTFE lined silicone septum	21.62	7.06	6.30	27.81	62.79
6[c]	Tenax	Permanent	[d]PTFE lined silicone septum	41.45	7.44	6.73	28.56	84.18
7	Tenax	Permanent	PTFE lined butyl septum	0.48	6.72	7.16	79.15	93.51
8	Tenax	Permanent	Swagelok	N.D.	7.52	7.18	77.35	92.05

[a] Setup 4 was repeated in a separate experiment with setup 6, [b] Additional petroleum ether and ethanol traps attached during sampling [c]Gas-tight application via septum over water phase, [d] Septums were cleaned in a dishwasher and reused, N.D. : no data

- Ethylene glycol (EG), used in previous study, was not effective to trap volatilised decalin.
- Tenax performed better when permanently attached. Additional volatile solvent traps connected to tenax (setup 5) did not improve recoveries.
- Gas-tight application via septum (setup 6) did not improve recovery (suggesting no immediate losses during application).
- PTFE lined butyl septum and Swagelok system did not act as an additional sink.

Conclusion

- Decalin is an extremely challenging substance to test under OECD 309 due to its volatile and hydrophobic properties.
- Partial separation and analysis of silicon oil was feasible by direct and indirect methods. Decalin shows a high affinity for silicon oil, since the majority of decalin partitioned to the silicon oil which would impact its bioavailability in degradation testing.
- Application solution prepared using passive dosing with ^{14}C labelled decalin had too less radioactivity (~12 Bq/mL) to conduct robust radioactive analysis, rendering passive dosing unfeasible for decalin testing within the OECD 309 TG.
- Ethylene glycol volatile traps were ineffective. Tenax trap were effective but would impact bioavailability with up to 79% of AR measured in traps. . Sorption of decalin to vessel components and glassware.
- The feasibility of OECD 309 for VHS substances such as decalin should be questioned.

References
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