

READY BIODEGRADABILITY TESTING OF VOLATILE POORLY WATER SOLUBLE FRAGRANCES; POSSIBLE PITFALLS

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ABSTRACT

Biodegradation is mediated by microorganisms participating in the recycling of organic compounds thereby preventing long residence times in the environment (persistence). Ready biodegradability tests described in OECD guidelines (301 series and 310) have been developed for water-soluble, non-toxic substances. Fragrance materials are often volatile and poorly water soluble and can, in some cases, be toxic to micro-organisms at the test substance concentrations used in OECD ready biodegradability tests. These properties may result in limited biodegradation in ready biodegradability tests and as a consequence, the substance being considered as potentially persistent. A justifiable outcome of ready biodegradability tests requires methodology preventing possible pitfalls such as adsorption of the fragrance substances on synthetic materials, inaccurate administration, inhibitory effects due to high initial concentrations and/or limited bioavailability. The use of "inert" septa and introduction of silicone oil in the test vessels will be illustrated with two fragrance substances.

INTRODUCTION

- Ready Biodegradability Tests (RBTs) & specified Inherent Biodegradability Tests (IBTs) are important screening test methods for assessing persistence (P) under the new European REACH regulation (table 1).
- Fragrance materials are often volatile, poorly water soluble and may in some cases, be toxic to micro-organisms at test concentrations used in OECD biodegradability tests.
- These factors can lead to false negative biodegradability results under standard prescribed conditions and to substances being considered as potentially P (table 2)
- A negative result in a RBT does not necessarily mean that the compound is persistent in the environment. Establishing for some materials (ready) biodegradability will require more comprehensive testing.
- Methods for improving the ready biodegradability testing of 2 fragrance materials (decanal and allyl cyclohexylpropionate) are described here. From their chemical structure, both materials are expected to degrade under environmental conditions.

Table 1: Examples of European REACH screening criteria for P

Type of data	Criterion	Screening assignment
Ready biodegradability test (OECD 301 series and 310)	Readily biodegradable ≥60% mineralization within 28 days	Not P & not vP
Specified test on inherent biodegradability MITI II test (OECD 302C)	≥70% mineralization (O ₂ uptake) within 14 days; log phase no longer than 3d; no pre-adapted inoculum	Not P & not vP

Table 2: Persistency screening assessment for 2 fragrance materials, biodegradability test results under standard prescribed conditions

	RBT Data		IBT Data	Screening (v)P criteria fulfilled?
	OECD 301F	OECD 310	OECD 302C	
decanal	42%	50%	66% (14 days) 78% (28 days)	Yes
allyl cyclohexylpropionate	56%		29% (14 days) 84% (28 days)	Yes

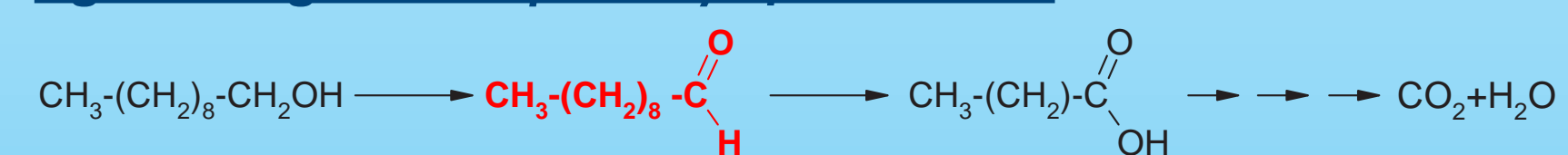
METHODS

The fragrance materials evaluated were decanal (CAS# 112-31-2) and allyl cyclohexylpropionate (CAS# 2705-87-5). Two types of tests were conducted: standard biodegradability tests according to the OECD 310 method (GLP compliant, carbon dioxide headspace test) and modified OECD 301D closed bottle test (non-GLP). Since the test materials at concentrations used in the OECD ready biodegradability tests could be toxic to the micro-organisms, the silicone oil was added for accurate administration and to decrease the inhibitory effects of the test material by enabling slow release from the silicone phase to the aqueous phase. In addition, because substances were suspected to adsorb onto the butyl rubber septa, minivert valves of Teflon[®] were used to close the test vessel.

Decanal

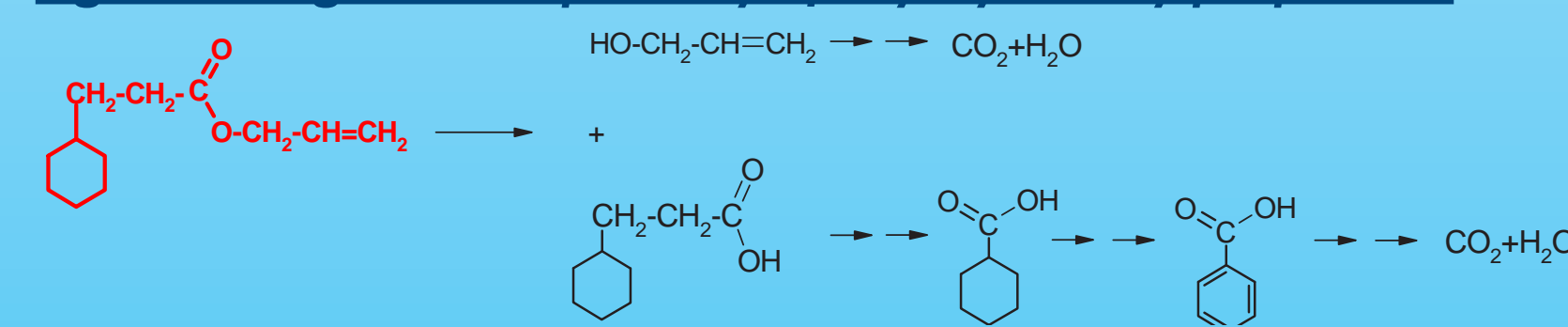
Decanal is an intermediate in the biodegradation pathway of 1-decanol (Figure 1). Both 1-decanol and decanoic acid are readily biodegradable.

Fig. 1 Biodegradation pathway of 1-decanol.



Allyl cyclohexylpropionate

Fig. 2 Biodegradation pathway of allyl cyclohexylpropionate

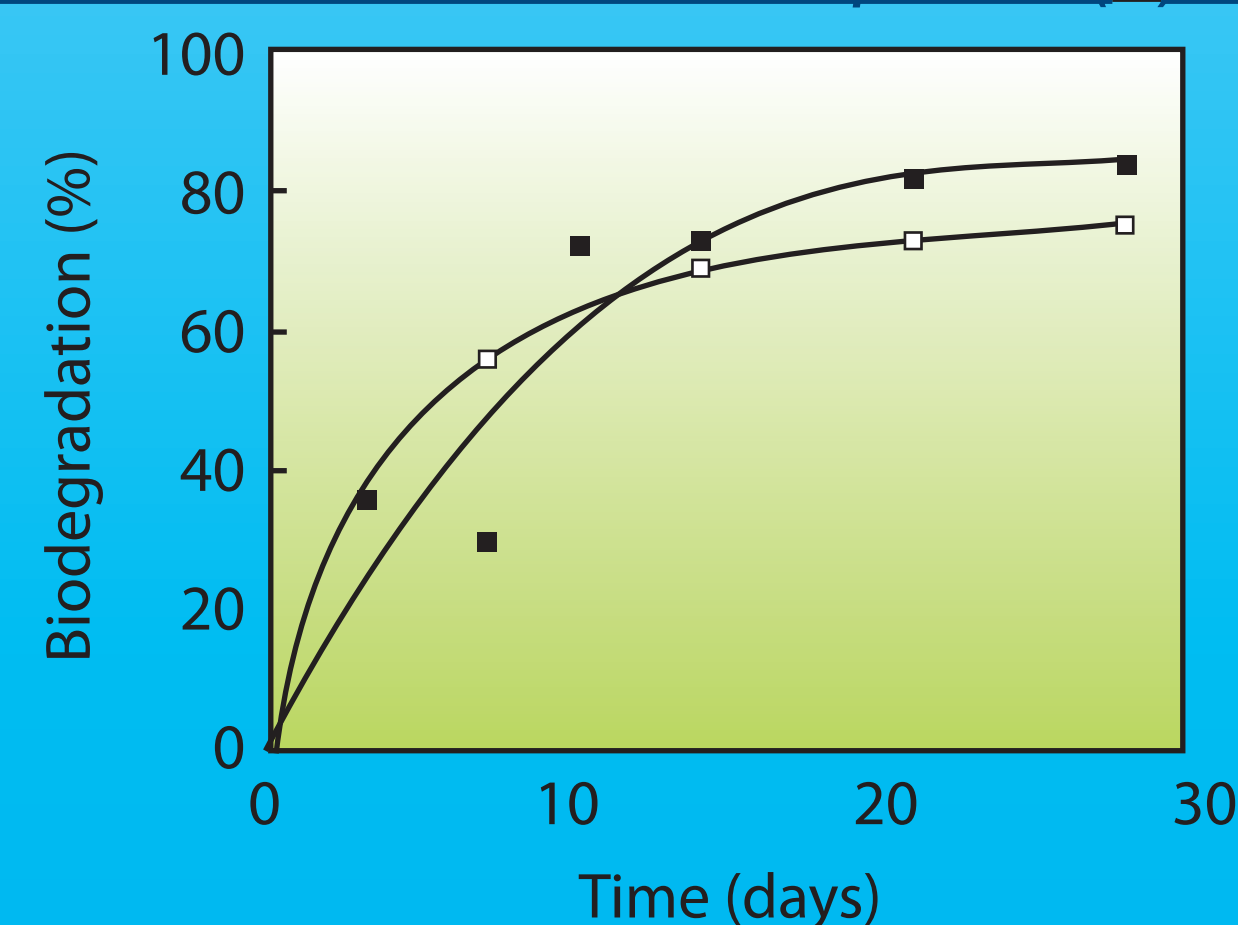


RESULTS

Decanal

- Carbon Dioxide Headspace Test: initial biodegradation of 25% achieved with butyl rubber; 72% biodegradation achieved using Miniert valves (Teflon)
- Closed Bottle Test: 66% biodegradation achieved at day 14 (second phase of silicone oil introduced to accurately administer test substance; glass stopper)

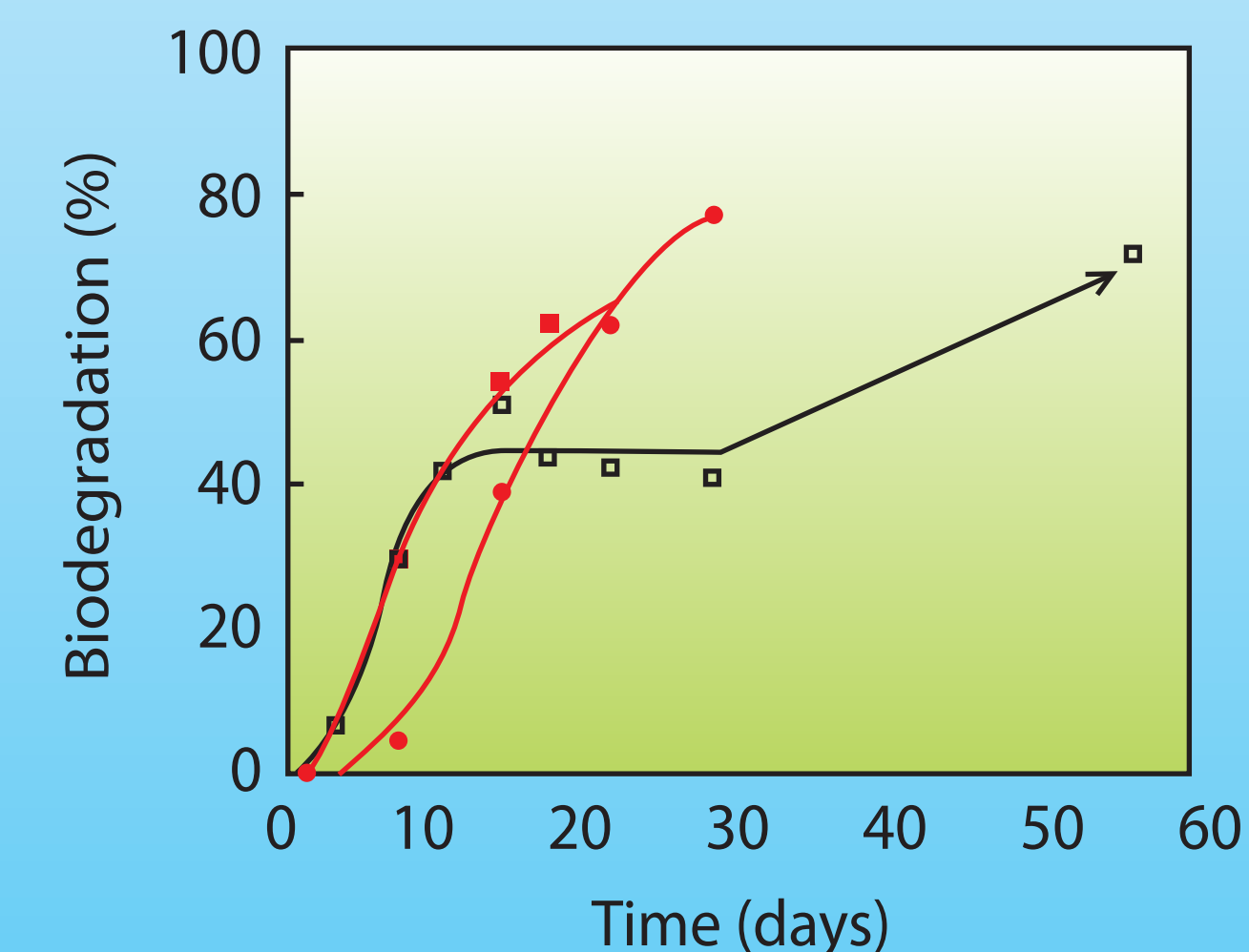
Fig 3. Biodegradation of decanal in the Carbon Dioxide Headspace test (■) and the Closed Bottle test (□).



Allyl Cyclohexylpropionate

- Carbon Dioxide Headspace Test: biodegradation of 41% reached at day 28; 71% at day 56 (Miniert valves)
- Carbon Dioxide Headspace Test (non-GLP): biodegradation of 78% at day 28 (second phase of silicone oil introduced to accurately administer test substance; glass stopper)
- Closed Bottle test: 64% biodegradation achieved at day 28 (second phase of silicone oil introduced to accurately administer test substance; glass stopper)

Fig 4. Biodegradation of allyl cyclohexylpropionate in the Carbon Dioxide Headspace test with (●) and without silicone oil (□). Silicone oil was also introduced in the Closed Bottle test to improve assessment of the biodegradability (■).



CONCLUSION

Decanal and allyl cyclohexylpropionate achieved ≥ 60% biodegradation in improved ready biodegradability tests (e.g. using inert materials and silicone oil). Both substances are confirmed as "not persistent".

Table 3: Persistency screening assessment for 2 fragrance materials, biodegradability test results under improved conditions

	OECD 301D	OECD 310	Screening (v)P criteria fulfilled?
decanal	66% (14 days) ^a	72% (28 days) ^b	No
allyl cyclohexylpropionate	64% (28 days) ^a	71% (56 days) ^{b,c} 78% (28 days) ^a	No

DISCUSSION

Volatile substances, like fragrance materials should preferably be tested in closed systems without synthetic material capable of adsorbing the fragrance substances. Carbon Dioxide Headspace tests (OECD 310) carried out with septa made out of Teflon[®] instead of the prescribed butyl rubber show the importance of the synthetic materials used in the test system. For poorly water-soluble substances, the specified high test substance concentrations are controversial because of the limited bioavailability of the test substance administered. These concentrations also lack environmental relevance for fragrance materials. The Closed Bottle test (OECD 301D) carried out with glass bottles and stoppers allows an initial test substance concentration of approximately 1 mg/L. The Closed Bottle test using continuous agitation is therefore probably the best method to assess the biodegradability of poorly water soluble compounds. A method to administer and possibly improve the bioavailability of fragrances to the competent microorganisms is essential. Biologically inert silicone oil allowed accurate administration in both the Closed Bottle test and the Carbon Dioxide Headspace test. "Slow" release prevents high initial test substance concentration potentially inhibitory to microorganisms in the biodegradability test.

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