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Current limitations of biodegradation screening tests and prediction of biodegradability: A focus on fragrance substances

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HIGHLIGHTS

- Biodegradation screening tests and predictions were analyzed for selected fragrances.
- Among 48 compounds, OECD 301C tests delivered more false negatives than 301F tests.
- Restriction to 28 days incubation in the OECD 301F and other tests was often limiting.
- Biodegradation potential of rose ketone and other fragrances was poorly predicted.
- Input of more realistic data from less stringent screening tests may improve models.

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ABSTRACT

Key parameters of OECD biodegradation screening tests were analyzed for impact on the assessment of biodegradation potential of selected fragrance compounds.

Comparison of official Japanese OECD 301C test results for 48 predominantly readily biodegradable chemicals with recent OECD 301F screening data demonstrated a significantly higher occurrence of false negatives potentially attributable to inoculum limitations for the former, while for the latter, restriction to 28 days was frequently limiting.

Influence of test concentration was studied for two groups of quaternary carbon-containing compounds under high (OECD 301F) and low (OECD 301D) concentrations and extended incubations. Ionones were generally ultimately biodegradable within 28 days or readily biodegradable, whereas damascones reached ultimate biodegradation in OECD 301D tests only and required extended incubations.

Predictions from Biowin and Catalogic models were analyzed for ionones, damascones and other selected structural groups of fragrance compounds. Comparison with previously published experimental data revealed significant differences in results between OECD screening tests, thus illustrating the shortcomings of currently available data collections and the potential risk of training prediction models with false negative results, particularly if used for models that are primarily based on data from one specific test. In addition, the analysis revealed specific limitations for prediction models that cannot take into account positioning of fragments within a structure. As a result, it appears that the presence of more than one unfavorable fragment usually does not allow reaching currently accepted threshold scores that would identify a given compound as biodegradable.

The outcome of the analyses of this study underlines the need to take into account results from OECD screening tests that are best adapted to physico-chemical

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properties of fragrances (hydrophobicity, volatility and inhibition potential at high test concentration) and conducted at reduced stringency compared to OECD guidelines (extended incubation owing to reduced bioavailability). Negative MITI database results should be critically reviewed, particularly for quaternary carbon-containing compounds. For future efforts aimed at designing more environmentally benign chemicals, the apparent underestimations will have to be overcome by providing more realistic experimental data that should subsequently be used to improve current biodegradation prediction models for better guidance.

1. Introduction

The Organisation for Economic Co-operation and Development (OECD) screening tests for the assessment of ready biodegradability (RBTs) are useful and essential tools to determine a chemical's potential to undergo biodegradation in the environment. They were developed as simple and inexpensive methods to identify those chemicals that are not expected to be of concern in terms of environmental persistence and have found extensive use in hazard and risk assessments worldwide. The current guidelines for the six OECD 301 series of tests were published in 1992 (OECD, 1992), and later followed by the introduction of the OECD 310 test (CO_2 headspace test) in 2006 (OECD, 2006). Ultimate biodegradation, i.e. mineralization of a carbon-containing test compound to CO_2, H_2O and biomass, is measured either by formation of CO_2, consumption of oxygen or removal of dissolved organic carbon (DOC) and indicated by reaching a pass level, usually 60% or 70%, depending on the method (OECD, 1992, 2006). In order to be readily biodegradable, the pass level has to be reached after 28 days of incubation and within 10 days counted from the onset of biodegradation at 10% (10-day window) (OECD, 1992). In recent years, limitations of OECD screening tests caused by the application of these strict criteria have been discussed in the literature (van Ginkel et al., 2008; Thouand et al., 2011; Kowalczyk et al., 2015). Within the context of the REACH chemicals legislation in Europe, this led to the introduction of new categories of less stringent screening tests, the so-called modified and enhanced RBTs which allow the use of lower test compound concentrations and extension of incubation to 60 days, amongst others (European Chemicals Agency, 2014a). Enhanced RBTs are currently restricted to use in Europe for persistence assessments (PBT and vPvB assessments) but cannot be used for classification and labeling (European Chemicals Agency, 2014a, 2013).

Fragrance compounds are a structurally diverse group of small molecules that exhibit challenging physico-chemical properties for testing in aqueous solutions due to the fact that they are generally hydrophobic and volatile. According to the guidelines, this usually reduces the choice of OECD tests to four (OECD 301C, D and F as well as OECD 310) because DOC removal is not suitable to measure mineralization of volatile compounds (OECD 301A and E), and the use of open systems with aeration (OECD 301B) is not recommended either (OECD, 1992). Despite the fact that suggestions for improvement of solubilization of hydrophobic test compounds were published by the International Organization for Standardization (ISO, 1995), little has been reported on how changing other, more basic parameters of RBTs and the use of modified or enhanced RBTs can influence results for fragrance compounds and how these results should ultimately be interpreted for PBT/vPvB and environmental risk assessments (Jenner et al., 2011; Seyfried and Boschung, 2014).

In the present report, we illustrate how the following key parameters i.e. inoculum, test duration, and test substance concentration can impact biodegradability screening assessments for fragrance compounds. To this end, different sets of ready and enhanced ready biodegradability data have been analyzed and compared to results from current prediction models. Finally, we propose possibilities of improvement for assessment of biodegradability and predictions.

2. Material and methods

2.1. Chemicals

All chemicals, including fragrance test compounds used for the biodegradation experiments, were of at least 90% purity.

2.2. Biodegradation tests

2.2.1. OECD 301F tests

The tests were carried out according to the OECD guideline 301F (OECD, 1992). Mineral media were prepared from stock solutions: 100 mL of solution A (8.50 g/L KH_2PO_4, 21.75 g/L K_2HPO_4, 33.40 g/L $Na_2HPO_4 \times 2H_2O$, 0.50 g/L NH_4Cl), 10 mL of solution B (27.50 g/L $CaCl_2$), 10 mL of solution C (22.50 g/L $MgSO_4 \times 7H_2O$) and 10 mL of solution D (0.25 g/L $FeCl_3 \times 6H_2O$) were mixed, the volume adjusted to 10 L with demineralized water and the pH adjusted to 7.4.

Activated sludge was collected from the sewage plant at Villette (Thônex, Switzerland), which treats predominantly domestic wastewaters. The sample was filtered on a polypropylene 149 μm pore size filter (Spectrum Laboratories, Rancho