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Review

Refinement of biodegradation tests methodologies and the proposed utility of new microbial ecology techniques

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ABSTRACT

Society's reliance upon chemicals over the last few decades has led to their increased production, application and release into the environment. Determination of chemical persistence is crucial for risk assessment and management of chemicals. Current established OECD biodegradation guidelines enable testing of chemicals under laboratory conditions but with an incomplete consideration of factors that can impact on chemical persistence in the environment. The suite of OECD biodegradation tests do not characterise microbial inoculum and often provide little insight into pathways of degradation. The present review considers limitations with the current OECD biodegradation tests and highlights novel scientific approaches to chemical fate studies. We demonstrate how the incorporation of molecular microbial ecology methods (i.e., 'omics') may improve the underlying mechanistic understanding of biodegradation processes, and enable better extrapolation of data from laboratory based test systems to the relevant environment, which would potentially improve chemical risk assessment and decision making. We outline future challenges for relevant stakeholders to modernise OECD biodegradation tests and put the 'bio' back into biodegradation.

Contents

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1. Introduction

Ever since *Silent Spring* (Carson, 1962) there has been widespread public concern about the use of chemicals and their possible impact on the environment. *Silent Spring* facilitated the ban of the pesticide DDT for agricultural use in 1972 in the United States (US EPA, 1975; Grier, 1982) and the onset of greater regulatory and public interest in the environmental consequences of chemicals. The beginning of the chemical regulatory era within the EU can most likely be traced back to the demise of the 'principle of microbial infallibility' which was based on the belief that, given the opportunity and favourable conditions, any organic chemical would biodegrade (Painter, 1974). Ironically, synthetic surfactants produced in the 1950s under the concept of 'better living through chemistry' (Copley et al., 2012) were found to produce unsightly foaming in conventional wastewater treatment plants, leading to the realisation that they were not being degraded during treatment. The 1950s and 1960s heralded an era of biodegradability testing development centralised predominantly around the aerobic biodegradability of synthetic detergents (Allred et al., 1964; Bunch and Chambers, 1967).

As our awareness of environmental issues associated with the use of chemicals has increased over the past 40–50 years, so has the scientific understanding and regulatory requirements. However, in parallel, the global chemical industry has grown rapidly since 1970. Global chemical output (produced and shipped) was valued at US$171 billion in 1970. By 2010, it had grown to $4.12 trillion (Davies, 2009). Pesticide use has increased worldwide by 36 fold in the last 45 years (1960–2005) (Zhang et al., 2011). Global use of pharmaceuticals led to 112% increase in prescription drug sales recorded between 2000 and 2008 (FDA, 2013) and the global use of home and personal care products has increased by 232% and 750% between 1998 and 2013, respectively (Euromonitor, 2013). These trends are likely to continue for the foreseeable future, as growth in emerging markets in South America, Africa and Asia continues.

Various regulations have been developed to assess the adverse impacts of existing and new chemicals in the environment. Hazard data are generated to assess the persistence, bioaccumulation and toxicity of chemicals. Exposure models and emission scenarios have been developed to predict the distribution and transport of chemicals in the environment. A key component of both hazard determination (persistence) and environmental risk assessment is the accurate estimation of the biodegradation of a chemical in the environment.

The degree to which any individual chemical will partition and persist in the environment is governed by a number of intrinsic and extrinsic properties/factors. These include solubility/hydrophobicity, octanol/water partition coefficient (K_{ow}), soil organic carbon/water partition coefficient (K_{oc}), dissociation constant (pK_a), as well as characteristics of the environmental compartment (e.g., organic carbon content, soil/sediment particle size and viable number of bacteria). Microbial biodegradation of chemicals is the major process that affects chemical persistence in the environment (Copley, 2009). Although several OECD biodegradation test guidelines have been established to determine chemical fate under different environmental scenarios, the prediction of chemical fate is still challenging (e.g. biodegradation studies conducted at environmentally relevant concentrations, laboratory to field extrapolation).

Since information on the fate and behaviour of chemicals is required to make an assessment of environmental exposure, it is crucial that chemical fate is determined in an accurate manner and more important to be predictive of degradation rates in the environment. However, so far the chemical industry, regulators and academia have encountered difficulties with extrapolating data from standardised laboratory tests into the environment due to discrepancies between test conditions and the complexity of environmental conditions. Studying microbial interactions and their functions within inocula helps to link processes associated with chemical biodegradation at the community level. Such knowledge can be essential to better understand chemical biodegradation in OECD tests and in the environment. Application of microbial ecology methods in existing test systems can be used to optimise new test guidelines. They may also have the potential in helping to develop strategies to improve the reproducibility of OECD tests.

In this review we provide an overview of the current test methods used to assess chemical biodegradation and discuss their limitations. A review of the current OECD tests is essential to outline the key issues. e.g., microbial inocula, chemical concentration, biodegradation pass levels. It will enable stakeholders (academia, industry and government) to understand the major concerns regarding current test results and identify knowledge gaps/questions. New methods can then be proposed to address these questions. Finally, we provide a summary of technological developments which could improve the capacity of biodegradation tests to provide more reliable predictions of biodegradation in the complexity of real environments.

2. OECD biodegradation tests

2.1. Historical aspects and principal design of OECD test

A range of methods for investigating biodegradation processes have been developed to predict the fate of chemicals in the environment. Most efforts have focused on the fate of chemicals in the aquatic environment, especially in wastewater treatment processes (Reuschenbach et al., 2003). Testing biodegradability under laboratory conditions aims to obtain a reliable prediction of the likelihood of the biodegradability of chemicals in the environment (Pagga, 1997). Over 30 years ago, in 1981, the OECD first published its guidelines for testing the biodegradation of chemicals. There have been several amendments and additions since, including updated methods for assessing ready biodegradability in 1992 (OECD, 1992) and introduction of the CO_2 headspace test (OECD 310, 2006). In 1990, a classification in accordance with the OECD was proposed (OECD, 2005). Three groups of tests were defined: (1) ready biodegradability (or screening), (2) inherent biodegradability and (3) simulation (Lapertot and Pulgarin, 2006). A list of reference chemicals for use as positive and negative controls in standardized biodegradability tests (Table 1) has been proposed by regulators and industry with an agreed set of properties and characterised set of biodegradability behaviour, which cover a range of environmental persistence and non-persistence (Comber and Holt, 2010). These chemicals group into bins (Table 1), which align with OECD tiered testing (Fig. 1) and show the relationships