

# Assessing chemical persistence for “difficult substances” using latest developments in biodegradation screening and simulation tests

Geneviève Deviller, Rémi Patoux, Marie-Hélène Enrici

Toxicological and Environmental Risk Assessment, HSE department, 20 rue Marcel Sembat 69191 Saint Fons, France

## Introduction

In order to assess the persistence of substances, biodegradation screening and simulation tests conducted according to standard guidelines are commonly required. However, these methods are not always applicable for “difficult substances” which are toxic to micro-organisms or low soluble. Additionally, recent results from research initiatives (e.g. CEFIC-LRi ECO11, 18, 24, 25 projects) have shown that the test conditions can have a major impact on the estimated biodegradation rates and the prediction of the degradation pathways. Therefore, adapting the test design taking into account the characteristics of the substances is a pragmatic approach that may drive the study outcome and improve the realism of the assessment of persistence. Different strategies adopted by Solvay are presented together with their impact on the biodegradation rates and their interpretation for different regulatory purposes.

## How to address toxicity to micro-organisms in biodegradation tests ?

### Impact of testing a lower concentration and non toxic metabolite in screening tests

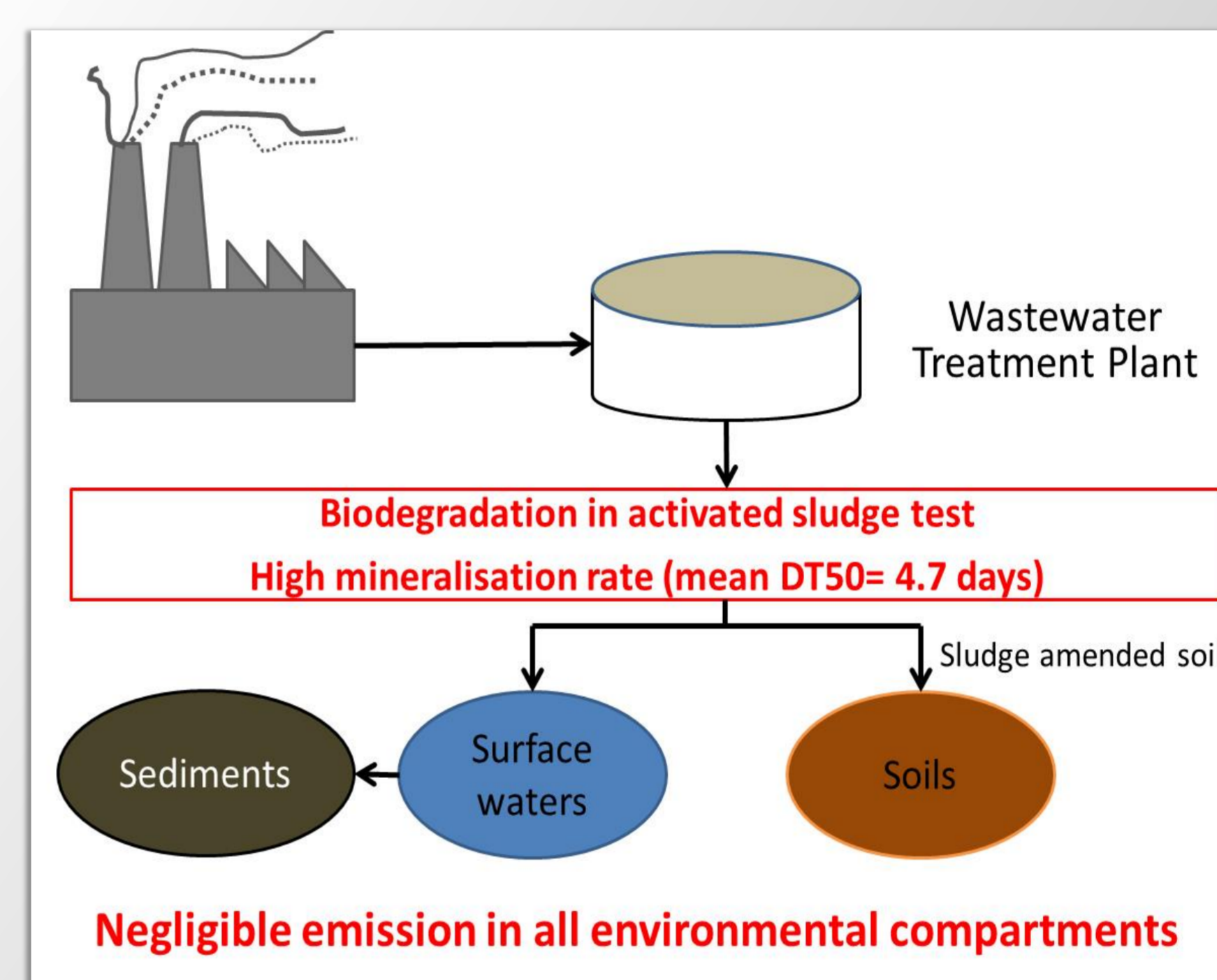
A substance toxic to the micro-organisms has been tested according to the OECD 306 guideline at the standard concentration (3 mg/L) and at a lower concentration (1 mg/L). In parallel, the same test has been performed on the environmentally relevant non-toxic metabolite of the substance (3 mg/L).

Biodegradation rate at 28 days	Toxic substance on micro-organisms at 3 mg/L	Toxic substance on micro-organisms at 1 mg/L	Non toxic oxidation product of toxic substance at 3 mg/L
Test item (%)	0%	11%	32%
Inhibition test (% of expected)	71%	96%	95%

The reduction of the testing concentration has improved the biodegradation rate by decreasing the toxicity of the substance to the micro-organisms as shown by the inhibition test results. The testing of the non-toxic metabolite has also increased the biodegradation rate for the same reason but at a higher extend. This may be due to an increased microbial growth that is generally assumed to be limited at low concentration and/or an enhanced bioavailability to the micro-organisms related to the structural difference (a double bond with oxygen instead of a single bond with carbon). These enhanced results will have no impact on risk assessment, Classification&Labelling nor PBT assessment but could impact the authorisation of products used in offshore under the Oskar convention.

### Biodegradation simulation test for a substance discharged in wastewater treatment plant

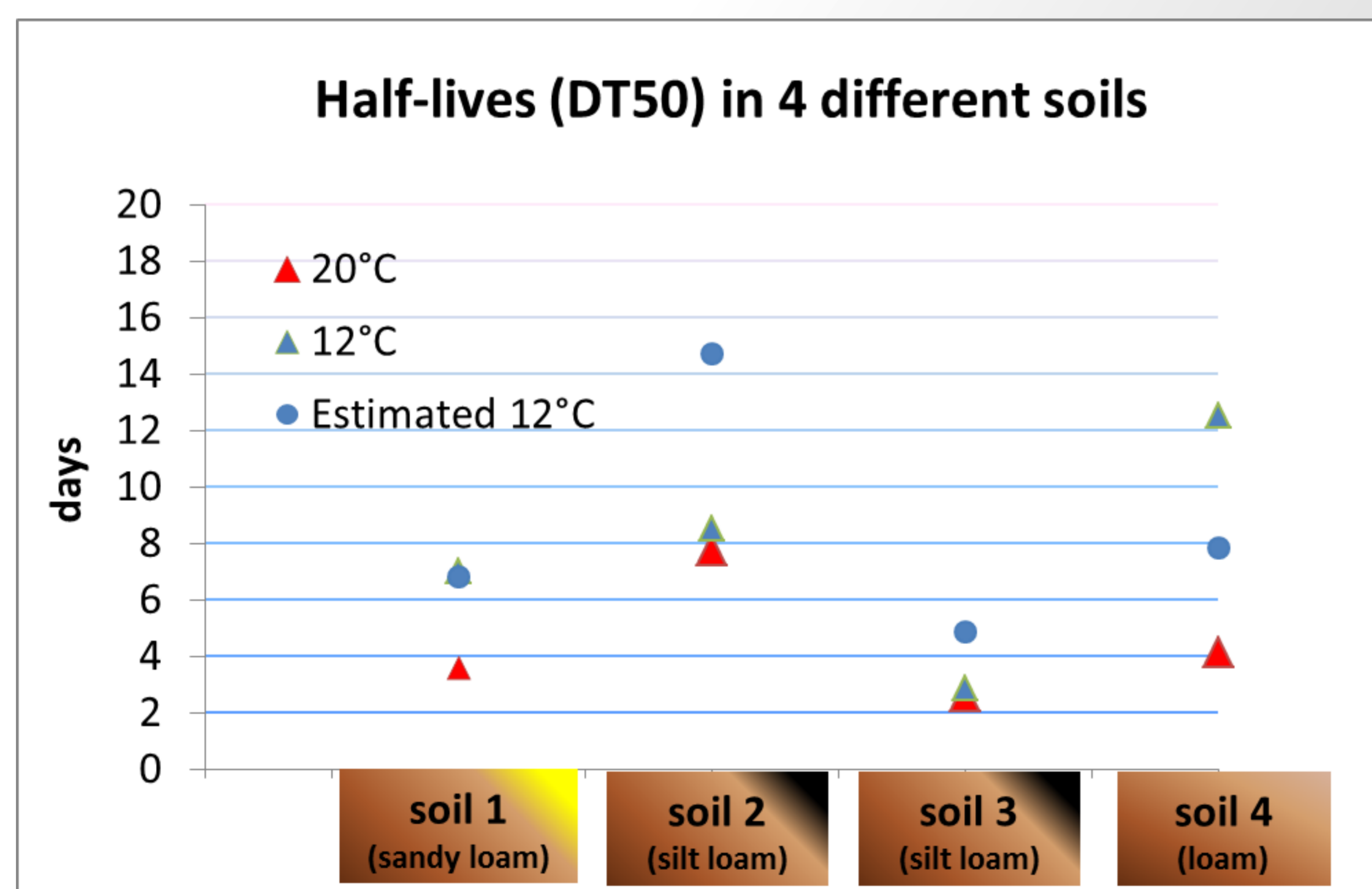
A substance considered not readily biodegradable (OECD 301D) and not inherently biodegradable (OECD 302B) due to its toxicity to the inoculum has been tested at low concentration according to a Sewage Treatment Plant (STP) simulation test (OECD 314B). For that purpose two radio-labelled samples on different carbon have been synthesised to assess the full mineralisation potential of the substance.



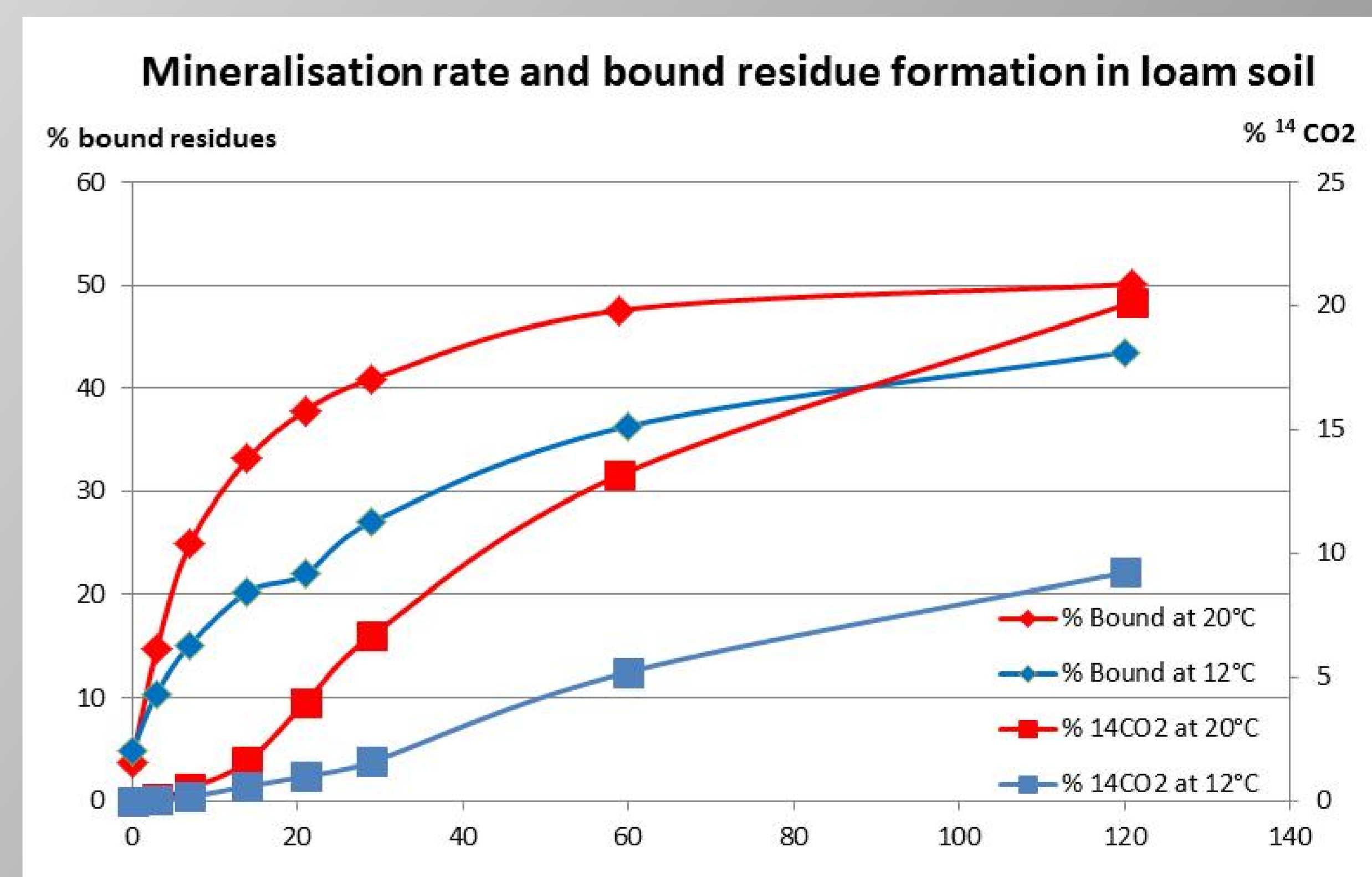
The results show that both radio-labelled substances are rapidly mineralised as CO<sub>2</sub> in STP (DT<sub>50</sub>= 2.6 and 6.7 days) and, STP being the first receiving compartment of the substance, a negligible emission in all environmental compartments was assessed together with a controlled risk (all RCR<1). We question whether this kind of data could be considered in PBT assessment of substances being systematically released through STP.

## How to address low soluble substances in biodegradation tests ?

The soil simulation test according to the OECD 307 test guideline is one possible method to investigate on the kinetics of degradation and the possible metabolites for the insoluble substances displaying a high capacity for adsorption. In addition, the choice of the test was also supported by the end uses of the substances which could not exclude releases to the soil compartment. This test was conducted on a “difficult” substance (solubility < 10 µg/L, LogK<sub>ow</sub> > 7, Log K<sub>oc</sub> > 5) at 2 different temperatures, in four European soils in order to define the influence of the new temperature of 12°C for degradation tests under REACH regulation on the degradation half-lives (DT<sub>50</sub>) and the formation of non-extractable residues.



**Figure 1:** For the two silt loam soils (2&3) the half-lives are identical at 12 and 20°C. For the sandy loam (1) and the loam(4) soils, the half-life is increased at 12°C compare to 20°C. Therefore, the impact of decreasing the testing temperature at 12°C is depending on the soil characteristics. It globally increases the half-lives variability among the soils and the persistence for regulatory interpretation. If we compare the experimental half-lives values obtained at 12°C with the estimated values using the Arrhenius equation (estimated 12°C), we observe that the results are consistent for only one soil (sandy loam) but underestimate (silt loams) or overestimate (loam) the biodegradation for the other soils.



Bound residues levels in the 4 soils were in the range of 39 – 51% at 12°C and 41 – 52% at 20°C after 120 days of experiment. There does not seem to be any evidence that bound residues would be more important at one temperature rather than another one.

**Figure 2:** In the loam soil where the kinetic of mineralisation is significantly higher at 20°C than at 12°C after 120 days (DT<sub>50</sub>=4,1 days at 20°C and 12,5 days at 12°C), there is not such a difference in the proportion of bound residues (51,5% at 20°C and 43,5% at 12°C). Then it can be concluded that performing tests at 12°C does not help decreasing bound residues.

**Conclusion:** Assessing biodegradation in the environment of difficult substances is a challenging domain due to the technical issues and their impact on the regulatory decisions. At Solvay, we have tested different conditions for toxic and low soluble substances using biodegradation screening and higher tier simulation tests. The results show that it is important to consider realistic exposure conditions (environmentally relevant released substance and receiving compartments) in order to comply with regulatory objectives in a proper way. In addition, the kinetic of degradation at different temperature seems to be related to the characteristics of the soil and testing at a lower temperature (12°C) does not help in decreasing the bound residues proportion.