Biodegradation **7:** 377–382, 1997.

Effect of concentration and environmental form of tetradecenyl succinic acid on its mineralization in soil

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Accepted 2 September 1996

Key words: bioavailability, builders, detergents, kinetics, mineralization, sewage sludge, soil

Abstract

Tetradecenyl succinic acid (TSA) is the major component of a detergent builder (C12-C14 alkenyl succinic acid), which is inherently biodegradable. ^{14}C-TSA was dosed as a component of sewage sludge into a soil with a history of sludge amendment at final added concentrations of 1.5 and 30 mg (kg soil)$^{-1}$. In addition, it was dosed to the soil in an aqueous solution to a final added concentration of 30 mg (kg soil)$^{-1}$. Dose and form were found to have a pronounced effect on the mineralization kinetics. When dosed in a realistic form and concentration (i.e. 1.5 mg (kg soil)$^{-1}$ as a component of sludge), TSA was mineralized at its highest rate and to its greatest extent, and the mineralization half-life was 2.4 days. When dosed at 30 mg (kg soil)$^{-1}$ as a component of sludge, mineralization began immediately, and the half-life was 23 days. In contrast, when dosed at this concentration in aqueous solution, the onset of mineralization was preceded by a 13 day lag period and the mineralization half-life was 69 days. Primary biodegradation and mineralization rates of TSA were very similar. Approximately, half the radioactivity was evolved as $^{14}CO_2$, while the remaining radioactivity became non-extractable, having presumably been incorporated into biomass or natural soil organic matter (humics). This study demonstrated that TSA is effectively removed from sludge-amended soils as a result of biodegradation. Furthermore, it showed the effect that dose form and concentration have on the biodegradation kinetics and the importance of dosing a chemical not only at a relevant concentration but also in the environmental form in which it enters the soil environment.

Introduction

To assess the environmental risk of a substance it is important to evaluate:

- its emission, route of entry and transport through the environment,
- its inherent properties including physico-chemical characteristics, (bio)degradability, and ecotoxicity,
- the ambient conditions of the receiving compartments.

In the case of consumer chemicals, the terrestrial environment has received less attention than the aquatic environment. Nevertheless, these chemicals may be introduced into the soil by leaking sewer lines, septic tanks, use of effluents for irrigation, and application of sewage sludge as a soil amendment. Soils may therefore serve as sinks or removal sites for these organic compounds.

According to EU-legislation, the amount of waste water sludge that may be applied per year is currently limited (EEC 1986) to 2000 - 3000 kg.(ha.yr)$^{-1}$ dry weight, although higher amounts are permitted if application is less frequent. The Technical Guidance Documents for the EU Environmental Risk Assessment of Existing Substances (EEC 1992; EEC 1993a,b; EEC 1994) propose an application rate of 5000 kg.(ha.yr)$^{-1}$ for the agricultural compartment and 1000 kg.(ha.yr)$^{-1}$ for grassland used for cattle grazing. For both the soil and sediment compartment, these Guidance Documents assume that biodegradation occurs only in the water phase, and default environmental half-lives for compounds which are readily and/or inherently

biodegradable have been proposed as a function of the sorption characteristics of the chemical. Depending on the sorption coefficient (Kd), which correlates with $\log P_{ow}$, half-lives for chemicals present in standard EU soils would range from 30 to 30,000 days at screening level, assuming 5% organic carbon in soil.

Sorption of chemicals can have a major impact on biodegradation. Nevertheless, the quantitative aspects of sorption on biodegradation have not been intensively investigated (Alexander 1994). Recently, the effect of the association of organic chemicals (surfactants) with different soil constituents on their subsequent mineralization in soil was studied by Knaebel et al. (1994). This study was carried out under realistic conditions where the biological, chemical and physical characteristics of a natural soil were largely preserved. Kinetic parameters differed largely as a function of the soil constituent to which the surfactant was associated, and differences in mineralization kinetics for sand and the different clay minerals were attributed to relative abundance of binding sites. The investigators concluded that the greater the affinity of a chemical for the environmental matrix, the lower the availability of a chemical to the degrading populations.

Degradation in soil has mainly focused on pesticides and to some extent also on the surfactants in household detergents (Kloepper-Sams et al. 1995). Our study assesses the role of bioavailability and matrix effects on the biodegradation of a moderately sorptive detergent builder, tetradecenyl succinic acid (TSA). TSA is the major component of a commercial alkenyl succinic acid builder. This material has an average adsorption coefficient (Kd) to activated sludge of 2200 $l.kg^{-1}$. Alkenyl succinic acid is rapidly and completely biodegradable in aquatic biodegradation tests after a short acclimation period, e.g. in a CO_2 production test. Measured removal in wastewater treatment plants is well in excess of 90% (OECD tests 301B, 302A and 303; Procter & Gamble, unpublished). Assuming that 50-60% of TSA is removed through sorption in a wastewater treatment plant (a typical range for a Kd of 2200 $l.kg^{-1}$), predicted exposure concentrations in soil at time of sludge application range from 1 to 3 $mg.(kg\ soil)^{-1}$.

The objectives of this study were to:

- determine the primary and ultimate biodegradation rate of TSA in a sludge amended soil, and
- compare the effect on mineralization kinetics of dosing the chemical as a neat material in aqueous solution versus adding it as a component of sewage sludge.

Materials and methods

Test chemicals

The ^{14}C-labeled material was pure [2,3-di-^{14}C]-2(2'-tetradecenyl) succinic acid (TSA) with a specific activity of 4.6 $\mu Ci.mg^{-1}$ and a radiochemical purity exceeding 99%.

Environmental samples

The soil was a clay-loam freshly obtained from an agricultural field in Arisdorf (Switzerland), which had been regularly amended with sewage sludge over the past 5 years. A sample was taken from the upper 20 cm during the summer of 1992. The soil had a pH of 7.43, an organic carbon content of 2.45%, and a cation exchange capacity of 22.59 $meq.100\ g\ soil^{-1}$. It consisted of 52.8% sand, 16.6% silt and 30.6% clay, The field capacity was 0.46, and maximum water holding capacity was 0.51. At the time of study initiation, microbial biomass equaled 101.3 $mg\ C.(100\ g\ soil)^{-1}$. Prior to its use, the soil was passed through a 2 mm sieve.

Activated sludge was obtained from a municipal waste water treatment plant in Sissach, Switzerland. The sludge was washed 3 times with tap water via centrifugation. At the time of the study, TSA was used in Switzerland, and hence the sludge and the soil had been pre-exposed to the test material.

Biodegradation testing

Each test system consisted of soil (100 g) in sealed 1 liter flasks, equiped with a lid with in- and outlets, and which were incubated at 20 ± 2°C in the dark. The headspace of each flask was continually flushed ($60\ ml.min^{-1}$) with CO_2-free air, which had been humidified in a gas washing bottle containing water. The effluent gas was sparged sequentially through two gas washing bottles containing 50 ml of 2N NaOH to absorb $^{14}CO_2$ and at the end a gas washing bottle with 50 ml of ethylene glycol to trap volatiles. Eight replicate flasks were started for the treatment where 30 mg $TSA.(kg\ soil)^{-1}$ was dosed as a mixture with sludge, and three replicates for the other two treatments.

The test chemical was dosed to the soil as a neat radiolabeled material dissolved in water or as a component of sewage sludge. In the former case, approximately 1 ml of water containing 3.0 mg (13.8 μCi) per ml of the test material was added drop-wise to 100 g

of soil. This represented a final added concentration of 30 mg.(kg soil)$^{-1}$. In the latter case, the test chemical was dosed to sludge, which was then mixed with the soil. Sludge (28.5 g) with a dry matter content of 3.5 % was mixed with either 1.5 mg (3.45 μCi) or 30 mg (69 μCi) of the test material and brought to 50 ml, resulting in sludge concentrations of 1.5 and 30 g/kg dry weight sludge, respectively. Five ml of these mixtures were added drop-wise to 100 g of soil. This dosing resulted in final added concentrations of TSA of 1.5 and 30 mg.(kg soil)$^{-1}$ and an added sludge concentration of 1 g sewage sludge per kg soil which represented a sludge application rate of 3000 kg dry matter ha^{-1}, assuming a 20 cm mixing zone and soil bulk density of 1500 kg.m^{-3}.

Following dosing of the test material, the soil in the test system was adjusted to 40% of its maximum water holding capacity. During the incubation period, the flasks were regularly weighed and water was replaced to maintain the target moisture content. Samples from the KOH traps were removed periodically, mixed with INSTA-GEL II cocktail (Packard) and analyzed for radioactivity using a Packard TRI-CARB 460 CD or TRI-CARB 2000 liquid scintillation counter to determine evolution of $^{14}CO_2$. In a similar manner, samples from the ethylene glycol traps were mixed with INSTA-FLUOR II (Packard) and analyzed to determine volatilized organics.

Characterization of residual radioactivity

Replicate flasks of the 30 mg/kg treatment dosed with sludge were periodically sacrificed for analysis of residual parent. Subsamples (50 g) of the soil were extracted three times with acidified (HCl) ethylacetate. This combined extract was analyzed for radioactivity by liquid scintillation counting and concentrated with a rotary evaporator. Additional extraction of the soil with 20% formic acid in acetonitrile, 20% formic acid in methanol, phosphate buffer (pH 7) and 0.2 M CaCl2/acetonitrile/phosphate (25:50:25) at pH 4 did not recover any significant additional radioactivity, and indicated that the acidified ethylacetate alone provided sufficient extraction.

The ethylacetate extract was reconstituted and spotted onto TLC plates (20 x 5 cm, 0.25 mm silica gel 60 F 254; Merck, Darmstadt, Germany), which were developed using the following solvent systems: chloroform/methanol/2-propanol/water/formic acid (80.5:14:2.5:2:1) chloroform/methanol/water formic acid (80:14:2:4), and toluene/acetone/formic acid (80:19:1).

The plates were scanned using a Berthold Automatic TLC Linear Analyzer (LB 2842) equipped with a data processing system. The detection limit was estimated to be 500 dpm, corresponding to approximately 49 ng of TSA. R_f values for authentic TSA in the various solvents systems were 0.5, 0.73 and 0.27, respectively.

The extracted soil residues were combusted in a stream of oxygen at 800°C with copper oxide as the catalyst. The liberated CO_2 was absorbed in 10 ml of methyl-cellosolve/ethanolamine (3:1), which was mixed with INSTA-FLUOR II cocktail and analyzed by LSC.

Kinetic analyses

Data describing the loss of parent and mineralization of TSA were fitted to various decay and production equations using nonlinear regression. Regression analyses were performed using Jandel TableCurve 2D (version 2.0) software (Jandel Scientific, Erkrath, Germany). The fitted functions included zero-order with and without a lag and three half order with and without growth. The equations that provided the best fit were identified based upon statistical considerations (r^2 and number of parameter estimates) and visual inspection of the fit and residuals.

Results and discussion

Figure 1 shows the disappearance of TSA parent, evolution of $^{14}CO_2$, and incorporation of radioactivity into non-extractable soil residue as a function of time for 30 mg TSA.(kg soil)$^{-1}$ dosed as a component of sludge. All data are expressed as % of the initially added LSC counts, and thus represent a mass balance for the labelled part of the TSA molecule. No significant level (< 0.1%) of volatile compounds was detected, and the total mass balances ranged between 84 and 100.1%, with a mean and standard deviation of 92.2 ± 4.5%. There was no discernible change in mass balance with time.

The amount of extractable radioactivity fell from over 90% initially to less than 5% after 100 days. Concurrent with this decline was a decrease in parent TSA, an increase in the level of non-extractable radioactivi-

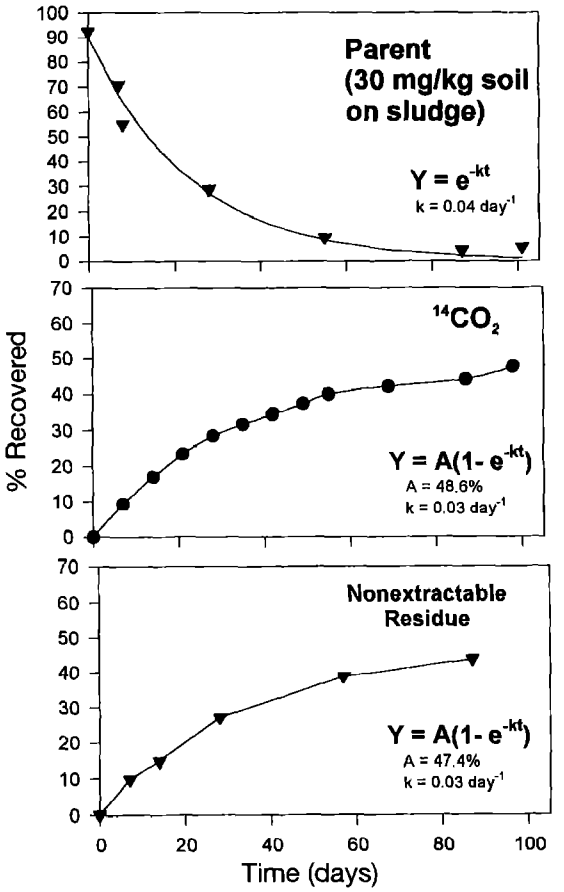

Figure 1. Recovery of parent, $^{14}CO_2$ and residual radioactivity as a function of time from ^{14}C TSA dosed as a component of sewage sludge into soil at a final added concentration of 30 mg/kg.

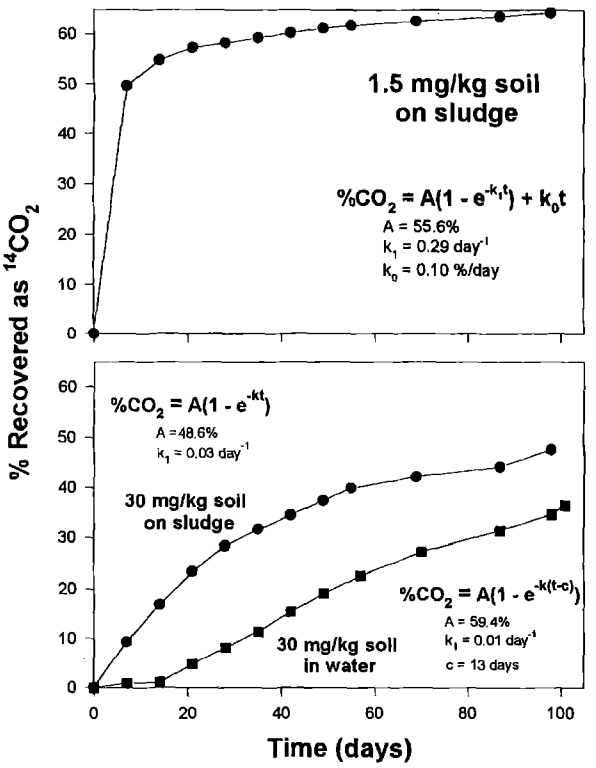

Figure 2. Evolution of $^{14}CO_2$ as a function of time from ^{14}C TSA dosed as a component of sewage sludge into soil at a final added concentration of 1.5 mg/kg (top panel), or dosed as a component of sewage sludge or in aqueous solution into soil at a final added concentration of 30 mg/kg (bottom panel).

ty in the soil, and evolution of $^{14}CO_2$. No extractable intermediates were observed. Disappearance of parent TSA could be accurately described by a first-order decay function. The first-order decay rate was 0.04 day^{-1}, which represents a half-life of 23.1 days. Evolution of $^{14}CO_2$ and incorporation of radioactivity into the non-extractable soil residue also exhibited first-order kinetics. The first-order rate constants for both processes was 0.03 day^{-1}, which was very similar to the decay rate for the parent. The similarity in the rate of primary biodegradation and mineralization indicated that these two sequential processes were in balance, which was consistent with the absence of significant levels of metabolite(s) in the TLC chromatograms. During biodegradation of the parent, approximately half the radioactivity was mineralized and the other half was converted to matter that could not be extracted using a variety of solvents. This non-extractable fraction was not further identified and was obviously tightly bound to some soil constituent or actually incorporated into the organic matter. This material likely represents radiolabeled carbon that was converted to biomass and/or natural soil organic matter (humics).

Figure 2 compares the mineralization of TSA as a function of concentration and dosing. Mineralization of 1.5 mg TSA.(kg soil) $^{-1}$ was more rapid and extensive than any other treatment. Notably, this treatment represents the most realistic dosing condition and concentration. Mineralization in this treatment was best described by a three-half order model without growth, as e.g. used by Brunner & Focht (1984). In this model, mineralization is biphasic in that an initial first-order phase is followed by a zero order process. The first-order rate constant was approximately 0.3 day^{-1}, which was ten and thirty times higher than the first-

order rates observed with the other treatments. The deflection point at which the process changed from first-order to zero-order occurred after ca. 14 days, at which point 56% of the initial radioactivity had been evolved as $^{14}CO_2$. Afterwards, approximately 0.1% additional radioactivity was evolved each day. It has been proposed that the first-order process describes the mineralization of the test chemical, while the zero-order process represents mineralization of the carbon incorporated into biomass or humics. Alternatively, it could be that the zero-order process represents the mineralization of a less bioavailable (e.g. tightly sorbed) fraction of the chemical.

The lower panel of Figure 2 shows the effect of dose form on the mineralization of 30 mg TSA.(kg soil)$^{-1}$. Mineralization of both treatments were best described by a simple first-order function. When dosed as a component of sludge, mineralization began immediately at a rate of 0.03 day^{-1}. In contrast, when dosed in aqueous solution, the onset of mineralization was preceded by a 13 day lag period and the mineralization rate was 0.01 day^{-1}. These rates represent mineralization half-lives of 23 days for TSA dosed with sludge and 69 days for TSA dosed in aqueous solution.

These results indicate that the presence of sludge had a profound effect on the mineralization of TSA in soil. The absence of a lag period in the presence of sludge suggests that sludge microbes remained active in the soil and were responsible for TSA mineralization. This conclusion is further supported by the very rapid mineralization observed in the 1.5 mg TSA.(kg soil)$^{-1}$ treatment, which corresponds to the sludge concentration at which sludge microorganisms were pre-adapted to in the field. The slower mineralization observed in the 30 mg TSA.(kg soil)$^{-1}$ is not surprising since this concentration is 20 times higher than that actually observed in the field. The lag period in the 30 mg TSA.(kg soil)$^{-1}$ dosed in aqueous suspension indicated that microbes capable of mineralizing TSA were indigenous to the soil but were at a low level, and time was required for them to reach to a level sufficient to mineralize the high dose concentration. It appears that addition of sludge supplemented this population. In addition to serving as an incoculum, sludge also had the potential to alter the bioavailability of TSA. In this study, the only evidence of reduced bioavailability was the lower rate observed when TSA was dosed directly to the soil. This lower rate, however, could also reflect different capabilities of the degrading populations in sludge versus soil. The asymptotic yields of CO_2 were similar for all treatments.

TSA is inherently biodegradable, and has a Kd of 2200 l.kg^{-1}. In a risk assessment for the soil compartment based upon the EU Technical Guidance documents for Risk Assessment of both New and Existing Chemicals (EEC 1993a,b; EEC, 1994), TSA would be expected to have a half-life of 3000 days. The latter estimate is 2–3 orders of magnitude higher than in reality, and is therefore appears highly conservative regardless of the dose or concentration tested.

Conclusions

Mineralization kinetics of TSA were comparable to those observed for surfactants, and half-lives ranged from 2.4 to 69 days. Dose concentration and form had a profound effect on the mineralization kinetics: when dosed in a realistic form and concentration (1.5 mg.(kg soil)$^{-1}$ as a component of sludge), TSA was mineralized at its highest rate and to its greatest extent. The calculated half-life was approximately 2.4 days. Primary biodegradation and mineralization rates of TSA were very similar. Approximately, half the radioactivity was evolved as $^{14}CO_2$, while the remaining radioactivity became non-extractable, having presumably been incorporated into biomass or natural soil organic matter (humics). When TSA was dosed as a component of sludge, it appeared that sludge microbes remained active in the soil and were responsible for an immediate onset of TSA mineralization. Nevertheless, dosing TSA directly to soil in water indicated that low levels of microbes, capable of mineralizing TSA, are indigenous in the soil.

This work highlights the importance of dosing a chemical to soil not only at a relevant concentration but also in the environmental form in which it enters the terrestrial environment.

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