Environmental Fate of Hexabromocyclododecane (HBCD)
The Degradation Pathway

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Introduction

Hexabromocyclododecane (HBCD) is currently undergoing risk assessment in the EU (Existing Substances Regulations 793/93/EEC). During the risk assessment process, the environmental persistence of HBCD was investigated. In previous studies (Davis et al 2005, 2006a), the biodegradation of HBCD was reported to occur in freshwater aquatic sediments and digester sludge. Using ^{14}C-labelled HBCD, it was demonstrated that HBCD was sequentially debrominated to t,t,t cyclododecatriene (t,t,t CDT). Moreover, the degradation rates of the three major diastereomers of HBCD (α, β, γ) were similar. The next step in elucidating the biodegradation pathway of HBCD was to investigate the fate of t,t,t CDT. Results from an initial experiment, based on a ready biodegradation test and conducted with t,t,t CDT, showed primary degradation of t,t,t CDT (Davis et al 2006b). However, due to the high background respiration (in relation to the low CDT concentrations of 1 mg to 10 mg/L), mineralization could not be verified. In the present study, the use of ^{14}C radiolabeled t,t,t CDT enabled to determine both the extent of mineralization of t,t,t CDT and the metabolites formed during the degradation.

Materials and Methods

[^{14}C]-t,t,t CDT was used in a test design based on key elements of the OECD 301B ready biodegradation test (OECD 1992).

Dosing CDT: [^{14}C]-t,t,t CDT was coated/dispersed on silica gel (35-60 mesh; Sigma-Aldrich Corporation, St. Louis, Missouri) to facilitate its addition to the reaction mixtures. t,t,t-[^{14}C]CDT was mixed (i.e. roller cell mixer) with the silica gel in sealed bottles, at nominal loadings of 1 mg and 0.2 mg [^{14}C]-t,t,t CDT per gram of silica gel, for at least 24 hours at approximately 20°C. The coated silica gel was used for the biodegradation experiment.

Experimental design: A measured volume of inoculated mineral medium and [^{14}C]-t,t,t CDT coated on silica gel were placed in vessels sealed with Teflon coated septa and mixed in the dark at 20 ± 2°C. The inoculum concentration was set at 30 mg/L mixed liquor suspended solids (MLSS). The experimental set up included viable reaction mixtures containing [^{14}C]-t,t,t CDT at two concentrations, 0.2 mg/L and 1mg/L. In addition, the following controls were included: heat sterilized controls (abiotic), medium blank controls, viability controls with aniline, and toxicity controls where the possible toxicity of t,t,t CDT was assessed by following aniline biodegradation. Selected reaction mixtures were sacrificed for analysis on days 0, 7, 14, 15, 21, 28, 35, 49, 50, 63 and 77.

Analyses: Reaction mixtures were extracted with acetonitrile and analyzed by high performance liquid chromatography with radiochemical detection (HPLC-RCD) to measure [^{14}C]-t,t,t CDT and other

[^{14}C]material. An Agilent 1100 series HPLC was used with a mobile phase containing 80% acetonitrile and 20% water delivered at 1.0 mL/minute. The [^{14}C]-t,t,t CDT was separated on a Phenomenex LUNA C8 column (4.6 x 150 mm, 5 µm particle size). Compounds eluting from the column were detected with a UV detector (195 nm) and a Berthold model LB–509 HPLC Radioflow Detector arranged in series. Under the described conditions, [^{14}C]-t,t,t CDT eluted with an approximate retention time of 10 minutes.

In separate reaction mixtures, $^{14}CO_2$ produced from the mineralization of [^{14}C]-t,t,t CDT was measured. The $^{14}CO_2$ assay involved acidifying the reaction mixture (pH<2) with phosphoric acid and sparging the mixtures with nitrogen gas (~250 cc/minute for 1 hour). The gas was passed through duplicate caustic traps to collect the $^{14}CO_2$. The contents of the two traps were combined and assayed by liquid scintillation counting (LSC).

Results and Discussion

The results are illustrated in Figure 1. Extensive biodegradation of [^{14}C]-t,t,t CDT was observed at both test concentrations during the study. Within 21 days, concentrations of [^{14}C]-t,t,t CDT were reduced to 65.3% of initial radioactivity for the 0.2 mg/L test mixtures and 77.1% of initial radioactivity for the 1 mg/L test mixtures. After 63 days, [^{14}C]-t,t,t CDT concentrations were reduced to 8.3% of initial radioactivity for the 0.2 mg/L test mixtures, while [^{14}C]-t,t,t CDT concentrations were reduce to non-detected levels (< 0.8%) after 77 days in the 1 mg/L test mixtures. In contrast, the concentrations of [^{14}C]-t,t,t CDT in the abiotic and mineral medium blank control mixtures showed little change during the duration of the study. Concentrations of t,t,t-[^{14}C]CDT in these mixtures ranged from approximately 86% to 98% of initial radioactivity at the conclusion of the study. Thus, the degradation observed for [^{14}C]-t,t,t CDT in the viable test mixtures was due to biological processes.

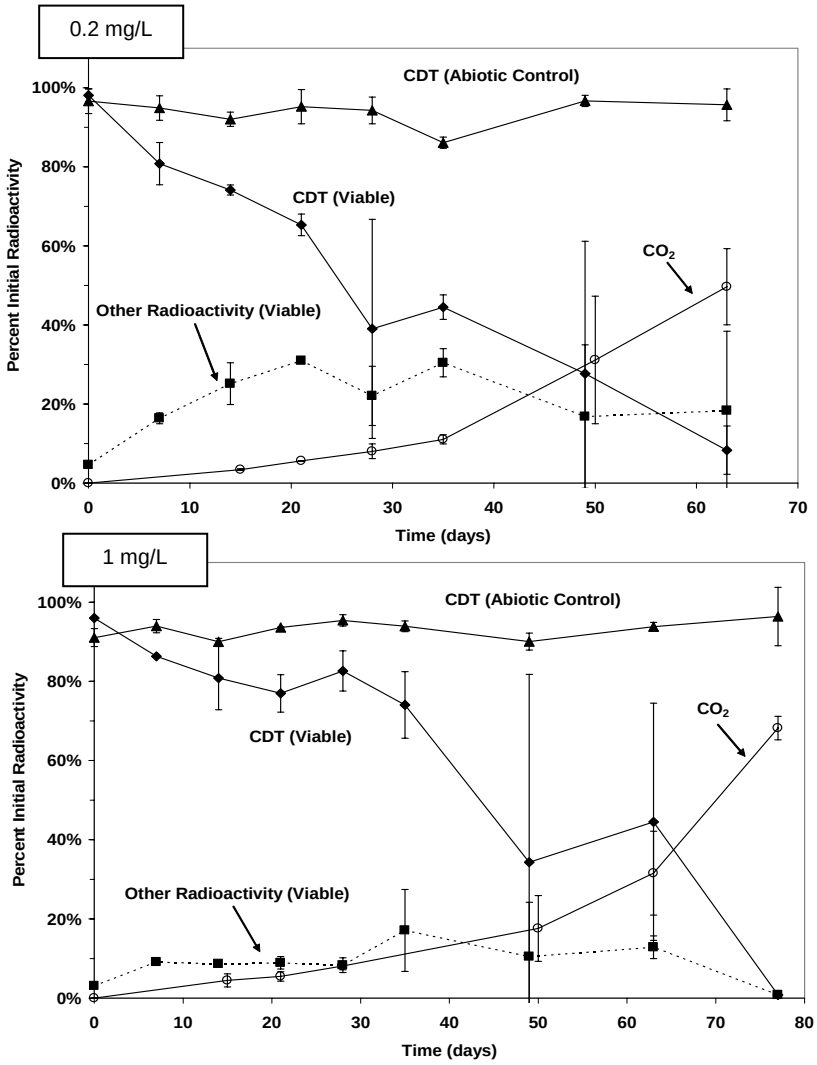
Extensive mineralization of [^{14}C]-t,t,t CDT to $^{14}CO_2$ was observed (Figure 1). Mineralization of [^{14}C]-t,t,t CDT reached 49.7% of initial radioactivity after 63 days in the viable 0.2 mg/L test mixtures and 68.3% of initial radioactivity after 77 days in the viable 1 mg/L test mixtures. In contrast, mineralization of [^{14}C]-t,t,t CDT in the abiotic controls at both test concentrations remained at 2.1% or less during the study.

Analysis of the viable test mixtures by HPLC-RCD typically showed radioactivity at retention times of ~ 10 minutes ([^{14}C]-t,t,t CDT), ~ 5 minutes (unidentified metabolite), and ~ 3.5 minutes (solvent front). The radioactivity associated with the "solvent front" was comprised primarily of $^{14}CO_2$. The "solvent front" radioactivity increased over time in the viable reaction mixtures, reaching 42.8% and 58.5% of initial radioactivity in the 0.2 mg/L and 1 mg/L reaction mixtures, respectively, consistent with the increased levels of $^{14}CO_2$ measured in the test mixtures. In contrast to the viable mixtures, "solvent front" radioactivity in the abiotic and mineral medium blank controls remained at 5.1% or less.

Radioactivity measured in the test mixtures which was not present as radioactivity in the solvent front or as [^{14}C]-t,t,t CDT, was designated as "other radioactivity". Most of this "other radioactivity" eluted as an unidentified metabolite at ~ 5 minute retention time. This "other radioactivity" was likely a transient intermediate produced from the degradation of [^{14}C]-t,t,t CDT. For both sets of viable reaction mixtures the levels of this material continued to increase during the first month of the study, reaching maxima concentrations equivalent to 30.4% and 17.1% of initial radioactivity for the 0.2 mg/L and 1 mg/L reaction mixtures, respectively. Thereafter, the levels decreased and by the end of the incubation time their concentrations were 18.3% and non-detectable for the 0.2 mg/L and 1 mg/L reaction mixtures, respectively.

The viability controls (aniline added) showed 97% degradation of the aniline (based on dissolved organic carbon removal) within 14 days, confirming suitability of the test conditions and a viable inoculum. The toxicity controls containing 0.2 mg/L and 1 mg/L [^{14}C]-t,t,t CDT showed complete degradation of the aniline within 14 days. Thus, [^{14}C]-t,t,t CDT at 0.2 mg/L and 1 mg/L was not inhibitory to the microbial inoculum under the conditions of the test.

Figure 1: Biodegradation and Mineralization of [^{14}C]-t,t,t CDT



Conclusions

In previous studies, it was demonstrated that HBCD was sequentially debrominated to t,t,t CDT. The next step in assessing HBCD's degradation pathway was to investigate the biodegradation of t,t,t CDT, under the stringent conditions of a ready biodegradation test. The results indicate that mineralization of t,t,t CDT can be achieved under aerobic conditions by microorganisms from wastewater treatment plants. This information added the last piece of puzzle to the elucidation of the HBCD degradation pathway. The first steps of degradation occur under anaerobic conditions, where HBCD can be sequentially debrominated down to t,t,t CDT. Then, under aerobic conditions, t,t,t CDT mineralizes to CO_2.

These results show that HBCD is not expected to persist in the aquatic environment. The major environmental sink for HBCD is the sediment, where anaerobic conditions prevail in the deeper layers The debromination of HBCD in this environment has been demonstrated. Based on the results shown in this study, it can reasonably be assumed that the formed t,t,t CDT will continue to degrade when exposed to aerobic conditions (e.g. as a result of water currents and turbulence), resulting in complete mineralization of HBCD.

References

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