Investigation of the Biodegradation of [^{14}C]Hexabromocyclododecane in Sludge, Sediment, and Soil

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Introduction

Hexabromocyclododecane (HBCD) is used to flame retard home and building insulation made of extruded or expanded polystyrene foam (primarily) and upholstery textiles as a back-coating.[1] HBCD may be released into the environment from production. HBCD is very hydrophobic and not readily biodegradable, and like other brominated flame retardants, has been presumed to be persistent in the environment. With increased attention directed towards control, reduction or elimination of discharges, emissions and losses of organic compounds, it is important to have a good understanding of the environmental fate and lifetime of HBCD.

Biodegradation, and abiotic processes such as hydrolysis and photo-oxidation, are important reactions that can bring about the degradation of organic compounds. Specifically, biodegradation can be one of the most important processes reducing the concentrations of organic chemicals in the environment.[2] Previous studies examined the lifetime of hexabromocyclododecane (HBCD) at realistic environmental concentrations under both aerobic and anaerobic conditions in authentic soil and aquatic sediment systems.[3] Biotransformation half-lives for environmentally relevant levels (ppb) of HBCD ranged from 7 to 63 days in soil and 2 to 32 days in sediments.[3] No brominated degradation products were detected.

The current laboratory study was performed utilizing Carbon-14 labeled HBCD ([^{14}C]HBCD) to facilitate the observation and subsequent identification of HBCD's degradation products. In addition, the relative degradation rates of the three stereoisomers (α, ß and γ) composing the commercial HBCD product were examined in all three environmental matrices (soil, sediment and sludge).

Materials and Methods

The transformation of HBCD was determined in municipal sludge, soil or water/sediment microcosms. For the sludge studies both aerobic (activated sludge) and anaerobic (digester sludge) reaction mixtures were prepared. For the aerobic studies the experimental approach followed the Organization for Economic Co-Operation and Development (OECD) Zahn-Wellens/EMPA test for assessing inherent biodegradability of compounds.[4] The anaerobic sludge studies followed the method outlined by the ISO Standard 11734 test for assessing the anaerobic biodegradability of compounds.[5] All sludge reaction mixtures contained [^{14}C]HBCD at a nominal concentration of 4 mg/L.

Anoxic reaction mixtures were prepared in an anaerobic atmosphere (~70% [vol] N_2, ~28% CO_2, and~2% H_2), sealed and incubated at 35 ± 2°C in the dark.

Soil and sediment studies were conducted according to the methods outlined by OECD Test Guidelines 307 and 308.[6,7] Aerobic soil and sediment microcosms were pre-incubated at 20 ± 1 °C and maintained by periodically exchanging the headspace of the microcosms with ambient air to replenish oxygen. Anaerobic sediment microcosms were prepared in an anaerobic atmosphere and were pre-incubated for approximately one month to allow the microcosms to stabilize.

[^{14}C]HBCD was added to each of the reaction mixtures at nominal concentrations of 4 mg/kg, which is approximately three orders of magnitude greater than the reported water solubility for γ HBCD (~3.4 μg/L). The [^{14}C]HBCD contains the α, ß, and γ stereoisomers of HBCD, similar in composition to test material used by Davis and coworkers.[3] Sufficient [^{14}C]HBCD was added to the reaction mixtures to ensure that [^{14}C]products formed at 10% yield (based on total radioactivity added) can be detected and that the degradation of each of the stereoisomers for the [^{14}C]HBCD (α, ß, and γ) can be followed through at least two half-lives.

The concentrations of [^{14}C]HBCD and [^{14}C]products in the different reaction mixtures were examined at selected time intervals. Reaction mixtures were extracted with acetonitrile for at least 16 hours and the distribution of radioactivity between [^{14}C]HBCD and [^{14}C]products was determined using reversed-phase high performance liquid chromatographic separation coupled with flow-cell radioactivity monitor (HPLC-RAM). Identification of [^{14}C]products formed at greater than 10% yield is being conducted using high performance liquid chromatography/mass spectrometry (LC/MS).

Results and Discussion

The aerobic and anaerobic degradation of [^{14}C]HBCD (~4 mg/L) was investigated in separate test systems using activated sludge (1,000 mg/L MLSS) from a domestic wastewater treatment plant (WWTP). Very little aerobic degradation was observed over 28 days. Conversely, rapid degradation of [^{14}C]HBCD was noted under anoxic conditions in the digester sludge (Fig 1) with the loss of approximately 50% of the [^{14}C]HBCD after 5 days. Degradation continued over the next 28 days with approximately 90% transformation of the ^{14}C-test material.

These results are consistent with the previous study by Davis and coworkers which reported much faster rates for the biotransformation of HBCD in aquatic sediments under low redox conditions compared to aerobic systems.[3] In the original study only the fate of the γ stereoisomer was followed, so that the degradation half-life was based on the disappearance of the γ stereoisomer. In the present study, the use of [^{14}C]HBCD allowed evaluation of the relative degradation rates of each of the three HBCD stereoisomers (α, ß and γ). The relative degradation rates in digester sludge of each of the three stereoisomers were approximately the same (Table 1). After 5 days, ~50-55% degradation was observed for all three stereoisomers under anaerobic conditions. By day 28, degradation of the α, ß, and γ stereoisomers were 87%, 89%, and 91%, respectively.

These results demonstrate that, in anaerobic digester sludge, the α, ß, and γ stereoisomers of HBCD are equally susceptible to biological degradation. Since the α and ß stereoisomers show a similar extent of disappearance (primary biodegradation) at different time points compared to the γ stereoisomer, a reasonable assumption is that the HBCD half-life based on the γ stereoisomer and determined in the original study[3] also applies to the α and ß stereoisomers.

In the present study, the degradation of HBCD resulted in the production of two major daughter products putatively listed as Product A and Product B (Figure 2). Product A was the major degradation product observed, and by day 7 represented over 50% of the initial radioactivity added to the digester sludge. By day 28, the level of Product A appeared to be declining and represented ~ 40% of the initial radioactivity. The second degradation product, Product B, constituted a minor degradation product and represented ~ 10% of initial radioactivity added to the test system. This level was reached after 7 days and little change in the percent levels of this component was noted over the next 3 weeks. Efforts are currently underway to identify these degradation products of HBCD.

Laboratory studies examining the degradation of [^{14}C]HBCD in soil and sediment systems are underway. Preliminary results indicate that degradation of HBCD is also occurring in the aerobic and anaerobic sediment reaction mixtures. Again, to facilitate identification of potential degradation products, the sediment reaction mixtures were dosed with HBCD at nominal concentrations (4 mg/kg) approximately three orders of magnitude greater than the reported water solubility for γ HBCD. Three major degradation products were noted in the anaerobic sediment reaction mixtures. Two of the degradation products in the sediment systems appear to be similar to Products A and B noted in the digester sludge (identical retention times on HPLC); see Figure 2). A third degradation product, Product C, was also observed in the acetonitrile extracts of the sediment reaction mixtures. Again, Product A appears to be the predominant degradation product in the anaerobic sediments.

Summary/Conclusions

Laboratory studies were initiated to identify degradation products of HBCD and to evaluate the relative degradation rates of the three HBCD stereoisomers (α, ß and γ) present in the commercial product. Aerobic and anaerobic reaction mixtures of soil, sediment or sludge were prepared containing ~4 mg/kg of [^{14}C]HBCD. Degradation of the α, ß and γ stereoisomers was noted in anaerobic digester sludge and both aerobic and anaerobic sediments. For the anaerobic digester sludge, the relative degradation rates of each of the three stereoisomers were approximately the same (half lives ~ 5 days). Several ^{14}C-degradation products were observed in the sediment and sludge reaction mixtures. Degradation Products A and B appeared to occur in both the digester sludge and aquatic sediments, while degradation Product C was only detected in the sediment systems. Efforts are currently underway to identify these degradation products.

References

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Figure 1: Percent radioactivity in acetonitrile extracts from anaerobic digester sludge

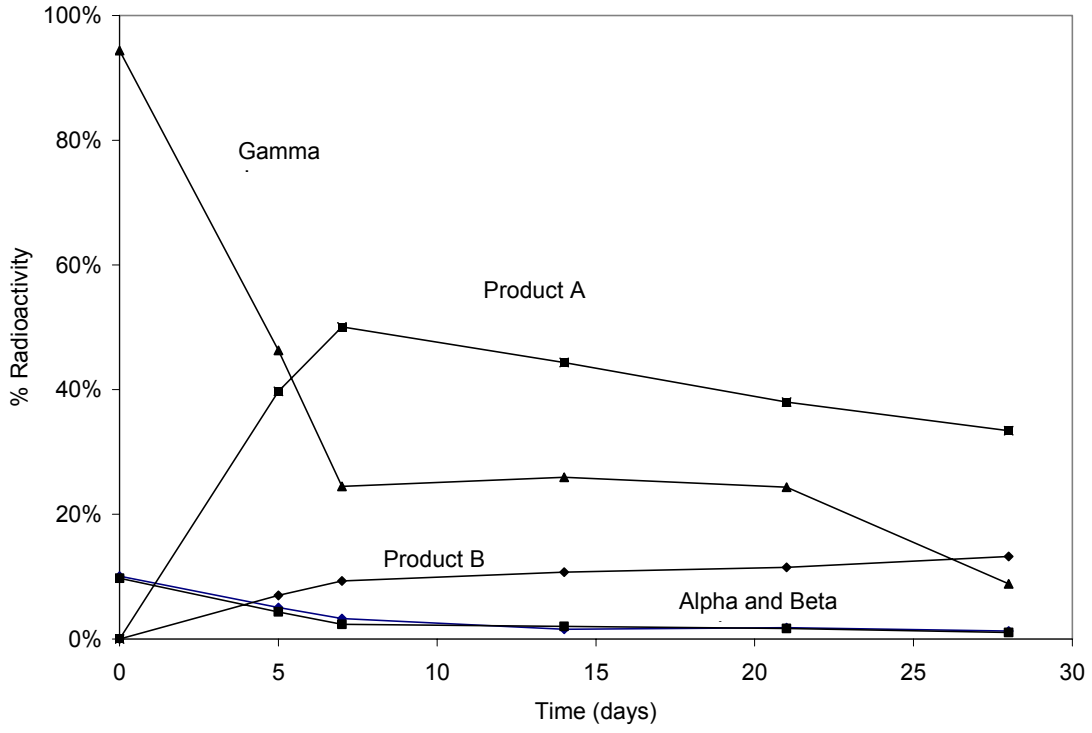

Figure 2: Chromatograms of acetonitrile extracts of anaerobic digester sludge: Reverse-phase high performance liquid chromatographic separation coupled with flow-cell radioactivity monitor (HPLC-RAM).

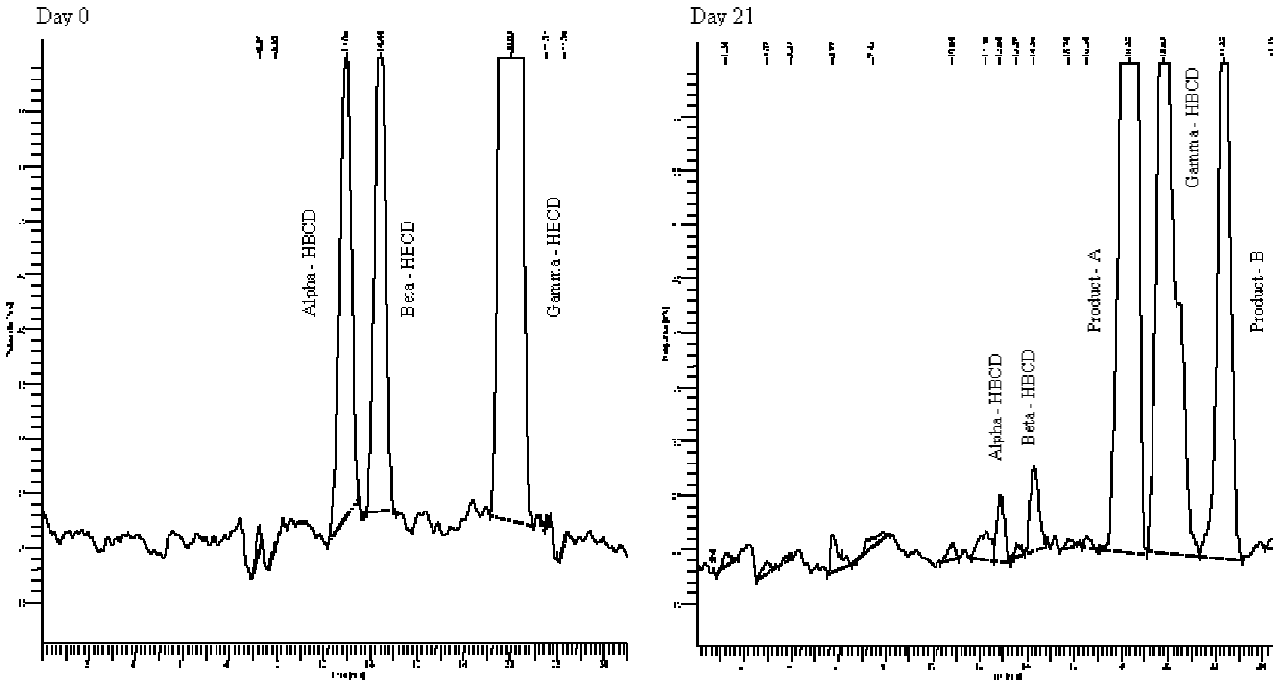

Table 1: Percent radioactivity distributed among the three stereoisomers of HBCD: Anaerobic digester sludge

Day:	0	5	7	14	21	28
α Isomer	10.1%	5.0%	3.3%	1.6%	1.8%	1.3%
% Degradation		***50%***	***67%***	***84%***	***82%***	***87%***
ß Isomer	9.7%	4.3%	2.4%	2.0%	1.7%	1.1%
% Degradation		**56%**	**76%**	**79%**	**83%**	**89%**
γ Isomer	94.4%	46.3%	24.5%	25.9%	24.4%	8.9%
% Degradation		***51%***	***74%***	***73%***	***74%***	***91%***