Fate and Effect of Hexabromocyclododecane in the Environment

René W. Hunziker[1], Stanley Gonsior[2], John A. MacGregor[3], Debbie Desjardins[3], John Ariano[4], Urs Friederich[1]

[1]Dow Europe, GmbH, Horgen
[2]The Dow Chemical Company, Midland
[3]Wildlife International, Ltd., Easton,
[4]Great Lakes Chemical Corp., West Lafayette

• Introduction

Hexabromocyclododecane (HBCD) is used as a flame retardant mainly in building insulation composed of extruded or expanded polystyrene foam. A minor use is in flame retardant back-coats of some upholstery textiles. Sales in Europe are estimated to be 9000 t/yr[1]. HBCD has been detected in a number of environmental samples mainly in sediment of urban areas[2, 3, 4]. In a series of acute aquatic toxicity tests, no effect was exhibited at concentrations equal to or below the water solubility of the technical product which consists of ca. 85% γ diastereomer[5]. However, considerable bioconcentration has been reported (log BCF=4)[5, 6]. In recent work it has been reported that a shift occurs along the food chain, from γ, the predominant isomer in the technical product, to the α isomer[3, 7, 8]. HBCD is very hydrophobic and not readily biodegradable, and has been presumed to be persistent in the environment. It is therefore important to have a good understanding of the environmental fate and lifetime of all HBCD isomers. This paper describes new findings on the water solubility of HBCD with respect to its 3 individual isomers, presents results on the acute toxicity in the marine alga *Skeletonema costatum* at the limit of solubility of all individual isomers and shows first data of an ongoing fate study with ^{14}C-HBCD where the primary biodegradation of the individual metabolites is differentiated.

• Material and Methods

A composite of commercial HBCD product from Albemarle Corporation, Dead Sea Bromine Group, and Great Lakes Chemical Corporation was used as test substance. The composite had a reported purity of 95.9%, and was composed of 8.0%, 5.4% and 86.6% of the α, ß and the γ diastereomers, respectively. Reference standards of the diastereomers were supplied by Great Lakes Chemical Corporation and Albemarle Corporation and had reported purities of 99.4%, 100% and 98.7%, for the α, ß and γ diastereomer, respectively. Reference standards were used to prepare combined calibration standards and concurrent matrix fortification samples. ^{14}C-HBCD was supplied by Wellington Laboratories, Inc., Guelph, ON Canada and was similar in composition to the technical product with 7.7% alpha, 7.8% beta, and 81.5% gamma isomers.

Water solubility has been determined at 20°C by means of a generator column according to OECD guideline 105[9]. The column was packed with Chromosorb W HP support material coated with the test substance that was prepared by mixing a Tetrahydrofuran stock solution with the support and drying at 35–40 °C. Reagent water was pumped through the generator column at 0.5 ml/min overnight to equilibrate the system and then set to a flow rate of 1 ml/min. After at least another 30 minutes, the eluate was collected drop wise directly into dichloromethane (DCM). Each eluate fraction was collected for 50 minutes (exactly 50.0 ml). Sample collection was repeated until five consecutive individual aqueous solute sample concentrations were within 30% of each other ('equilibrium'). Subsequently, the flow rate was lowered to 0.5 ml/min and again samples were collected until five consecutive individual aqueous eluate sample concentrations were within 30 % of each other, and within 30 % of the mean saturation concentration obtained with the first flow to confirm equilibrium conditions.

Toxicity to the marine algae *Skeletonema costatum* (diatom) has been determined in a 72 hour exposure test in saturated saltwater media (i.e., at the limit of solubility) at 20 °C and a 16 lighting and 8 hours darkness cycle. Saturated saltwater test media was prepared by means of the generator column technique following a procedure similar to the one outlined for the water solubility experiments. This technique provides an optimal exposure of the test substance to the water as equilibrium concentrations were established in the eluate for each of the isomers. Nominal algae concentration at test initiation was approximately 77 000 cells/ml. Samples were collected every 24 hours and algal cell densities were determined by means of an electronic particle counter. Pooled subsamples were examined microscopically for atypical cell morphology (e.g., changes in cell shape, size or colour). HBCD concentrations were determined from samples at the beginning of the test and at test termination. Growth in the treatment group was compared to growth of a negative control (media passed through generator column with uncoated packing) and a media control (media without further treatment) in terms of cell density, area under the growth curve and growth rate. Test procedure followed the ISO guideline 10253:1995**Fehler! Verweisquelle konnte nicht gefunden werden.**10.

Transformation of HBCD was determined in municipal sludge, and in soil and water/sediment microcosms under aerobic and anaerobic conditions using ^{14}C-HBCD. 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) could be detected and that the degradation of each of the isomers could 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.

Transformation of HBCD in sludge was investigated in both aerobic (activated sludge) and anaerobic (digester sludge) reaction mixtures. For the aerobic studies the experimental approach followed the OECD 302 B guideline[9]; the anaerobic sludge studies followed the ISO Standard 11734 test[10]. ^{14}C-HBCD was added 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.

Transformation of HBCD in soil/sediment microcosms were conducted according to the methods outlined by OECD Test Guidelines 307 and 308[9]. 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.

Analytical method consisted of an extraction to an organic solvent and separation and quantification with an LC/MS. In the transformation studies, ^{14}C-HBCD and ^{14}C-products were

separated and quantified by HPLC-RAM. Selected extract samples were further concentrated and LC/MS was used to identify ^{14}C-products present. In case of the algae toxicity studies and the water solubility studies, the extraction step consisted of partitioning twice to dichloromethane with subsequent rotary evaporation to dryness. Dry extracts were reconstituted with tetrahydrofuran-water (50:50 %) and submitted to LC/MS analysis. In case of the transformation studies, microcosms were extracted with acetonitrile. Extracts were filtered and submitted to LC/MS analysis.

- **Results and Discussion**

The water solubility of HBCD α, ß, and γ diastereomers in non-buffered reagent water was 48.8, 14.7, and 2.08 µg/L, respectively, and total solubility (i.e. their sum) was 65.6 µg/L. The difference in solubility between the γ and the α isomer is remarkable (more than an order of magnitude). The same difference in solubility was observed in various pre-tests and confirmed in the saltwater media (see below).

Algae toxicity: Mean concentrations, Day 0, in the saturated saltwater media (triplicate samples) were 34.3, 10.2, and 1.76 µg/L, for the α, ß, and γ HBCD diastereomers, respectively. The mean measured total HBCD concentration at study initiation and termination was 46.3 and 35.7 µg/L, respectively. The arithmetic mean total exposure concentration was 41.0 µg/L.

Figure 1 is a plot of the mean cell density over time. Exponential cell growth occurred in the control replicates (box plot, Figure 1). After 72 hours of exposure, cell density, biomass and growth rate in the 41.0 µg/L HBCD treatment group was 81, 79 and 92.7 %, respectively, relative to the Media Control. Cell density, biomass and growth rate in HBCD treatment group was 69, 69 and 89 %, respectively, relative to the Negative Control. There were no signs of adherence of cells to the test chambers or aggregation/flocculation of algae or noticeable changes in cell morphology in the controls or in the treatment group after the exposure. The inhibition of growth rate of 7 and 11 % relative to the media control and to the negative control, respectively, are statistically significant according to a Dunnett test and are considered to be treatment related. An inhibitory effect of 10% is generally considered as No Effect Level. The 72 hour EC50, based on cell density, biomass and growth rate was >41.0 µg/L total HBCD.

Aerobic and anaerobic transformation of ^{14}C-HBCD (~4 mg/L) was investigated in separate test systems using activated sludge (1 000 mg/L MLSS) and digester sludge (2 000 mg/L) from a domestic wastewater treatment plant. In the aerobic system very little degradation was observed over 28 days. Conversely, rapid degradation of ^{14}C-HBCD was noted under anoxic conditions in the digester sludge (Figure 2) 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. Degradation of HBCD resulted in the production of two major daughter products referred to as A and B. 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 (~ 10 % of initial radioactivity after 7 days, with little change over the next 3 weeks).

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 (nominal concentrations 4 mg/kg d.w.). 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 above based

on identical HPLC retention times. A third degradation product, Product C, was also observed in the acetonitrile extracts of the sediment reaction mixtures. Product A appears to be the predominant degradation product in the anaerobic sediments. Efforts are currently underway to identify these degradation products of HBCD.

In the following the presented findings are discussed in the context of the available data on HBCD. No acute toxicity of HBCD was found in a series of acute toxicity tests with daphnids (*Daphnia magna*) fish (*Lepomis macrochirus*, *Leuciscus idus L.*, and *Onchorhyncus mykiss*) and algae (*Scendemus subspicatus*, *Selenastrum capricornutum*) at the limit of solubility of the technical product[5]. Conversely, Walsh and co-workers have reported an EC50 of 9–12 µg/L in a 72 hour test with *Skeletonema costatum*[11]. The findings of the current work, a worst case exposure scenario with all three HBCD isomers at saturation, contrast the reported EC50 of Walsh et al. Whether or not comparable HBCD diasteroemer concentrations up to the level of saturation and predominance of the α isomer are reached under environmental conditions is questionable. Available data on levels in the environment are ambiguous. Isomer composition in sediments seems to reflect the technical product[3] whereas levels in the water phase are generally below the detection limit. Based on the reported increased level of the α isomer in biota[3,7,8] one can not directly conclude that higher levels of this isomer are present in the water phase. Isomer specific processes during uptake, metabolism and excretion might influence the ratio of the isomer in biota.

Regarding the findings on degradation, substantial primary degradation was observed for all three isomers in anaerobic digester sludge and both aerobic and anaerobic sediments. Very little degradation of ^{14}C-HBCD was observed over 28 days in aerated reaction mixtures containing 1 000 mg/L of activated sludge or in aerobic soil. These results are consistent with the previous study by Davis and coworkers[12] where they reported much faster rates for the biotransformation of HBCD in aquatic sediments under low redox conditions. 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 isomers (α, ß and γ). For the anaerobic digester sludge, the relative degradation rates of each of the three isomers were similar (half lives ~ 5 days). These findings do not indicate differences in the transformation behaviour of the three isomers.

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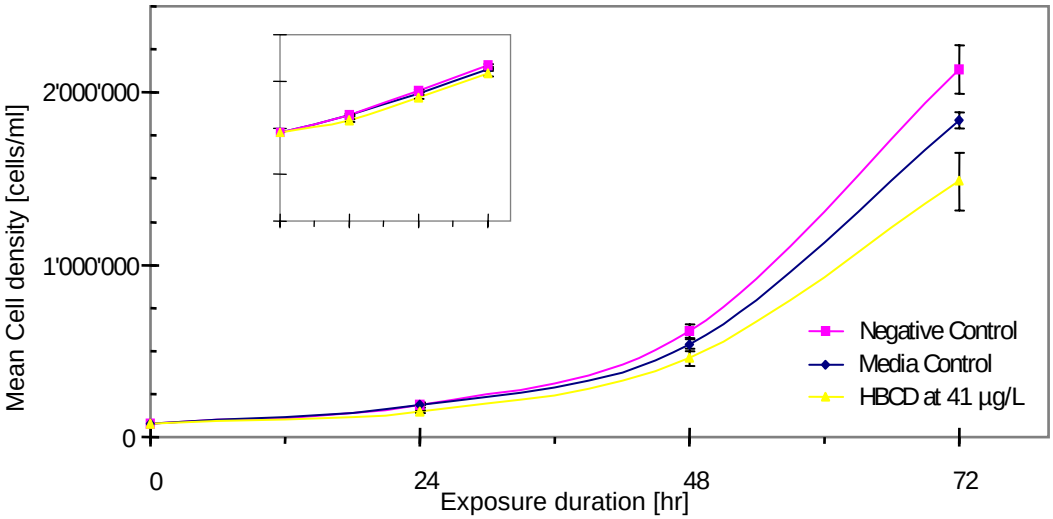

Figure 1: Mean cell density over time in negative control (saltwater media which has passed a generator column packed with uncoated support material, Chromosorb W HP); in media control (untreated saltwater media), and HBCD at 41 µg/L (saltwater media which has passed a generator column packed with HBCD coated support material). Box plot shows the same data on logarithmic scale.

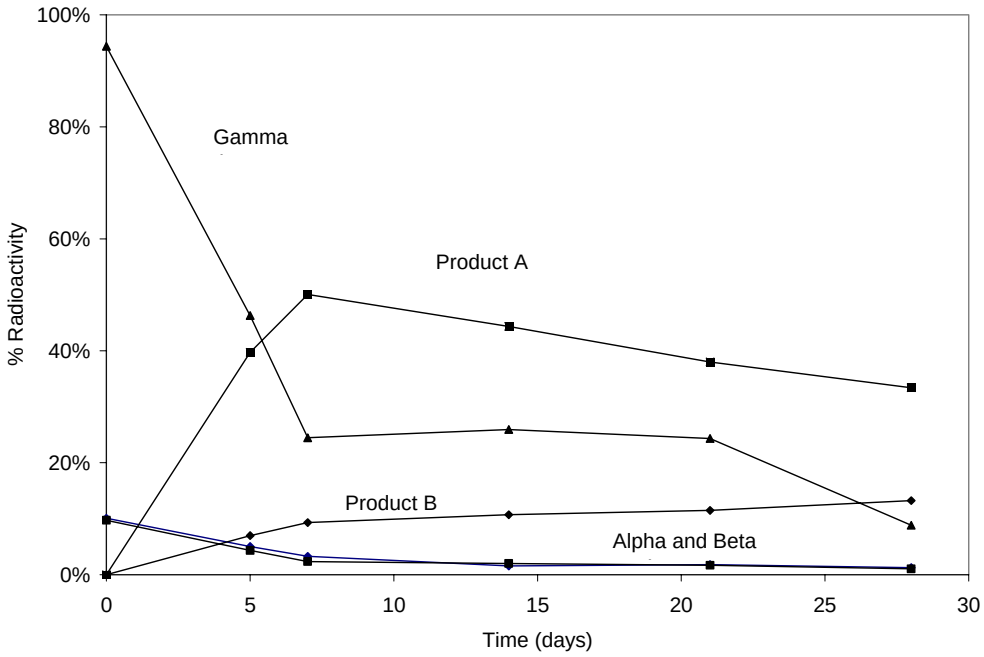

Figure 2: Percent radioactivity in acetonitrile extracts from anaerobic digester sludge.