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2,5,6,9,10-Pentabromocyclododecanols (PBCDOHs): A new class of HBCD transformation products

Norbert V. Heeb [a,*], Daniel Zindel [a,b], W. Bernd Schweizer [c], Peter Lienemann [b]

[a] *Swiss Federal Institute for Materials Science and Technology (Empa), Laboratory of Analytical Chemistry, Überlandstrasse 129, CH-8600 Dübendorf, Switzerland*
[b] *Zurich University of Applied Sciences, Institute of Chemistry and Biological Chemistry, Reidbach, 8820 Wädenswil, Switzerland*
[c] *Swiss Federal Institute of Technology (ETH), Laboratory for Organic Chemistry, Hönggerberg, 8093 Zürich, Switzerland*

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ABSTRACT

Pentabromocyclododecanols (PBCDOHs) are potential environmental transformation products of hexabromocyclododecanes (HBCDs). They are also potential stage one metabolites of biological HBCD transformations. Herein, we present analytical evidence that PBCDOHs are also constituents of technical HBCDs and flame-proofed polystyrenes (FP-PSs). PBCDOHs are possibly formed during the synthesis of technical HBCD, presumably during the bromination of cyclododecatrienes in aqueous isobutanol together with isobutoxypentabromocyclododecanes (iBPBCDs), which have been identified in these materials recently. Of the 64 stereoisomers possible, eight pairs of enantiomers, named α-, β-, γ-, δ-, ε-, ζ-, η-, and θ-PBCDOHs were separated with a combination of normal-, reversed- and chiral-phase LC. Crystal structure analysis revealed the stereochemistry of the α-PBCDOH pair of enantiomers, which was assigned to (1S,2S,5R,6S,9S,10R)-2,5,6,9,10-pentabromocyclododecanol and its enantiomer. Mass spectrometric data are in accordance with the expected isotope patterns. On a C_{18}-RP-column, the polar PBCDOHs eluted before the HBCD and iBPBCD classes of compounds. PBCDOHs were also found in FP-PS materials. The stereoisomer patterns varied considerably in these materials like those of HBCDs and iBPBCDs. Expanded polystyrenes were rich in late-eluting stereoisomers, similar to technical HBCD mixtures. Extruded polystyrenes contained more of the polar, faster-eluting isomers. The presented chromatographic and analytical methods allow a stereoisomer-specific search for PBCDOHs in biota samples, which might have experienced metabolic HBCD transformation reactions. Besides this potential source, it has to be recognized that PBCDOHs are by-products in technical HBCDs and in flame-proofed polystyrenes. Therefore, it is likely that PBCDOHs and iBPBCDs are released to the environment together with HBCD-containing plastic materials.

1. Introduction

Polystyrenes (PSs) are high production volume chemicals produced at more than 10 million t y^{-1} (Wünsch, 2000). They are used in form of expanded, low-density polystyrenes (EPSs) extruded, high-density polystyrenes (XPSs) and some other specialty materials such as styrene–butadiene and acrylonitrile–butadiene–styrene copolymers (ABS). These light-weight polymers are widely applied as insulation materials in the construction sector. For safety reasons, polystyrenes are flame-proofed for these applications. Hexabromocyclododecanes are currently the most important flame retardants for this purpose (Alaee et al., 2003). HBCDs are mixed with the polymer material but are not covalently bound to it like tetrabromobisphenol, a reactive brominated flame retardant, which is integrated into the polymer chain. Therefore, it is not unlikely that HBCDs may escape from such materials.

HBCDs are now ubiquitous environmental pollutants and their widespread occurrence has been documented on local, regional, and global scales (de Wit, 2002; Remberger et al., 2004; de Wit et al., 2006; Law et al., 2006; Covaci et al., 2006). HBCDs are considered as persistent (P), bioaccumulating (B) and toxic (T) and are therefore included in the PBT-list of the European Chemicals Bureau (ECB, 2010). HBCDs have been identified in humans (Weiss et al., 2004; Thomsen et al., 2005, 2010). They are frequently found in wildlife samples (Law et al., 2003; Sellström et al., 2003; Gerecke et al., 2003; Lindberg et al., 2004; Tomy et al., 2004; Peck et al., 2008) and in environmental compartments such as lake-, river-, and marine-sediments (Sellström et al., 1998; Marvin et al., 2006; Kohler et al., 2008), even in remote areas (Bogdal et al., 2010). Their properties have been reviewed thoroughly by the Persistent Organic Pollutant Review Committee of the Stockholm Convention and a decision is expected by the Conference of Parties in May 2012.

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* Corresponding author. Tel.: +41 58 765 4257; fax: +41 58 765 4614.
E-mail address: norbert.heeb@empa.ch (N.V. Heeb).
URL: http://www.empa.ch (N.V. Heeb).

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Transport of HBCDs into the environment is still a matter of debate. They may be released directly from industrial plants, where HBCDs and HBCD-containing polymers are produced and processed. The widespread use of these materials and subsequent degradation and disintegration is another potential HBCD source. The role of plastic debris as a carrier for such compounds in the aquatic environment has also been recognized (Moore et al., 2001; Derraik, 2002; Thompson et al., 2004; Browne et al., 2008).

Any time along this transport, transformation of HBCDs may occur. Early occasions are the industrial HBCD synthesis itself and the production and processing of HBCD-containing plastic materials. It was reported that technical HBCD mixtures also contain isobutoxypentabromocyclododecanes, which are possibly formed during the bromination of cyclododecatrienes in aqueous isobutanol (Heeb et al., 2010a). iBPBCDs were also identified in various flame-proofed polystyrenes together with HBCDs (Heeb et al., 2010b). Their patterns vary substantially for EPS and XPS. We showed that various isomerization reactions occur during thermal treatment of such materials (Heeb et al., 2008a,b, 2011). These isomerizations are HBCD transformation reactions too. They are fast, in the order of minutes, at temperatures above 110 °C, indicating that HBCDs and structurally-related compounds react at elevated temperatures. It is not clear yet, if such isomerizations are also possible at lower temperatures, e.g., under abiotic environmental conditions.

We hypothesized that hydroxylation reactions may also occur during industrial synthesis of HBCDs forming PBCDOHs. Analytical evidence is now presented that PBCDOHs indeed are constituents of technical HBCD mixtures and flame-proofed polystyrenes. But PBCDOHs are also potential environmental transformation products formed by metabolism of HBCDs. Hydroxylated HBCD metabolites have been observed lately (Brandsma et al., 2009; Esslinger et al., 2011). The findings presented here show that PBCDOHs are present in technical HBCD mixtures and may be released from flame-retarded plastic materials.

2. Materials and methods

2.1. Materials

Individual PBCDOH diastereoisomers were isolated from a low-melting, technical grade HBCD mixture (Saytex HP-900®, mp = 168–184 °C) by normal phase liquid chromatography (LC) on silica 60 (Merck, Darmstadt, Germany) with mixtures of *n*-hexane (Merck) and dichloromethane (Merck). HPLC-grade methanol (ROMIL, Cambridge, UK), acetonitrile (ROMIL), and water (Merck) were used for reversed- and chiral-phase LC. The examined plastic materials, a low-density EPS board ($\rho = 19$ kg m^{-3}) and a high-density XPS board ($\rho = 48$ kg m^{-3}) were obtained from different construction sites.

2.2. Sample purification and crystal growth

2.2.1. Isolation of diastereoisomers

A dichloromethane solution of a technical HBCD mixture was adsorbed on activated silica (SiO_2, F60, 230–400 mesh). The suspension was homogenized, dried with a stream of nitrogen, and loaded on activated silica and fractionated by normal-phase LC with *n*-hexane and various *n*-hexane/dichloromethane mixtures (Supplementary material). Sampled fractions were investigated with LC-MS for the presence of PBCDOHs.

2.2.2. Isolation of enantiomers and crystal growth

Fractions containing identical PBCDOH diastereoisomers were combined, concentrated to dryness, and dissolved in acetonitrile/water. Aliquots (60 μl) of filtered solutions were separated on a permethylated-β-cyclodextrin LC column (PM-β-CP, 200 mm × 4 mm, 5 μm, 100 Å, Nucleodex 5, Macherey-Nagel) with acetonitrile/water under isocratic conditions (48% acetonitrile). Fractions containing α-PBCDOH enantiomers were combined and concentrated at 50 °C in a stream of nitrogen. Upon cooling, crystallization occurred. The recovered material was suitable for X-ray diffraction analysis.

2.2.3. Preparation of polystyrene samples

Aliquots of the polystyrene samples were dissolved in dichloromethane and concentrated to dryness. Acetonitrile was added to the residues resulting in suspensions of polystyrene materials and clear supernatants. Aliquots of the supernatants were concentrated to dryness and dissolved in methanol/water (80/20) for LC-MS analysis.

2.3. Chromatographic characterization

Separation of different PBCDOHs was succeeded by a combination of normal- (SiO_2, F60, 230–400 mesh), reversed- and chiral-phase liquid chromatography (Spectra System P4000, Thermo Separation Products, San Jose, CA, USA). A C_{18}-reversed-phase column (C_{18}-RP, 125 mm × 4 mm, 5 μm, 100 Å, Nucleosil 100–5, Macherey-Nagel, Oensingen, Switzerland) with a methanol–water gradient (80% methanol for 5 min, 80–98% in 15 min, 98% for 4 min) at a flow rate of 1 ml min^{-1} was used to separate diastereoisomers. Separation of enantiomers was achieved with a permethylated-β-cyclodextrin chiral-phase column (PM-β-CP, 200 mm × 4 mm, 5 μm, 100 Å, Nucleodex 5, Macherey-Nagel) at a flow rate of 0.9 ml min^{-1} with a methanol/water gradient (75–85% methanol in 20 min, 85–98% in 10 min, 98% for 3 min). Samples were dissolved in methanol/water (80:20) and injected at aliquots of 20 μl. Identical chromatographic conditions were used to separate PBCDOHs, iBPBCDs and HBCDs. Therefore, respective data sets are comparable and can be used as reference (Heeb et al., 2010a,b).

2.4. Mass spectrometry and X-ray diffraction analysis

Mass spectrometric analysis of the LC-effluents was performed on a triple stage quadrupole mass spectrometer (TSQ 7000, Thermo Finnigan, San Jose, CA, USA). Sensitivity was higher with atmospheric pressure chemical ionization (APCI) than with electrospray ionization. PBCDOHs were detected in selective ion monitoring mode (SIM), recording prominent anions of the chloride adduct cluster $[M + Cl]^-$ at m/z 612.7, 614.7 and 616.7 at a corona current of 5 μA, an octapol potential of 0 V and heated capillary and vaporizer temperatures of 150 and 400 °C, respectively. A limit of detection at a signal-to-noise ratio $s/n = 3$ of 0.2 ng (on column) was determined for α-PBCDOH standards. The crystal structure measurement and data reduction was performed on a Bruker APEX-II Duo single crystal diffractometer with Mo-K_α radiation ($\lambda = 0.071073$ nm) equipped with a graphite monochromator (Bruker, 2010). Atomic coordinates and crystallographic details have been deposited with the Cambridge Crystallographic Data Center, deposition number CCDC 858571.

3. Results and discussion

3.1. Stereochemistry of PBCDOHs and their structural relation to HBCDs

PBCDOHs like iBPBCDs can be considered as first-generation transformation products of HBCDs resulting from a substitution of one of the six bromine atoms with a hydroxy- or an isobutoxy-group. Because all three classes of compounds are found in