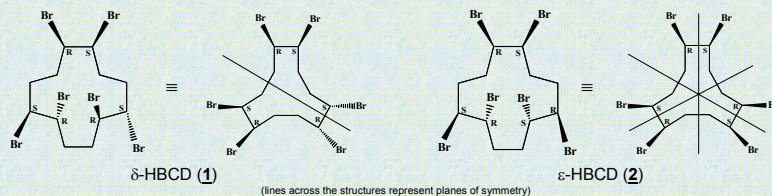


## Introduction

- Commercial hexabromocyclododecane (HBCD) consists largely of three diastereomers,  $\alpha$ -,  $\beta$ -, and  $\gamma$ -HBCD
- Recently, the presence of small amounts of two minor diastereomers,  $\delta$ - and  $\epsilon$ -HBCD, has been reported in the HBCD technical mixture.<sup>1</sup>
- These minor isomers have also been reported in environmental samples.<sup>2,3</sup>
- The present work has confirmed the identity of the two minor isomers in the HBCD mixture as being **1** and **2**.



## Experimental

**Synthesis of  $\delta$ - and  $\epsilon$ -HBCD**: Trans,trans,trans-1,5,9-cyclododecatriene (ttt-CDT) was brominated and the reaction mixture was cleaned up by chromatography and crystallization to give  $\delta$ - and  $\epsilon$ -HBCD.

**NMR Experiments**: <sup>1</sup>H NMR spectra were recorded at 400 MHz on a Bruker Avance DPX 400 NMR spectrometer.

**GC/LRMS**: Experiments were conducted on a Shimadzu GC/MS-QP2010 using a J&W 30m DB-5 column (0.25 mm ID, 0.25  $\mu$ m film).

**LC/MS**: Experiments were conducted on a Waters Acquity Ultra Performance LC interfaced to a Micromass Quattro micro API mass spectrometer. Separations were carried out on an Acquity UPLC BEH C18 column (1.7  $\mu$ m, 2.1 x 100 mm). A typical LC method started at 65% (80:20 MeOH/ACN) and 35% water (both with 10 mM NH<sub>4</sub>OAc) at a flow rate of 350  $\mu$ L/minute. The program was then ramped to 80% (80:20 MeOH/ACN) over 17 minutes and held for two minutes before returning to initial conditions. MS data for the molecular clusters [five isotopomers (m/z 636.5, 638.5, 640.5, 642.5, 644.5)] of the HBCD isomers were obtained in selected ion recording (SIR) mode.

## Results and Discussions

### Analysis of technical cis,trans,trans-1,5,9-cyclododecatriene (ctt-CDT)

- GC-MS and <sup>1</sup>H NMR analysis of a technical sample of ctt-CDT indicated the presence of approximately 3% ttt-CDT.
- Bromination of ttt-CDT should only produce two isomers having the structures **1** and **2**.
- Therefore, it is reasonable to assume that bromination of technical ctt-CDT should produce HBCD containing **1** and **2** as minor components.

### NMR Characterization of **1** and **2**

- Bromination of ttt-CDT gave two products.
- 1** and **2** are designated as  $\delta$ - and  $\epsilon$ -HBCD, respectively, on the basis of their elution order from a C18 column ( $\delta$  eluting before  $\epsilon$ ).
- Their NMR spectra confirm that  $\delta$ - and  $\epsilon$ -HBCD have structures **1** and **2**, respectively.
  - $\epsilon$ -HBCD (**2**) has a very simple <sup>1</sup>H NMR spectrum (Fig. 1a) due to the presence of three planes of symmetry in the structure. All six CHBr moieties are therefore equivalent giving a single resonance.
  - $\delta$ -HBCD (**1**) shows three non-equivalent CHBr moieties in its <sup>1</sup>H NMR spectrum (Fig. 1b) when it is recorded at 85°C due to one plane of symmetry for the average conformation.
  - The <sup>1</sup>H NMR spectrum of **1** becomes more complex (Fig. 1c) at a lower temperature because it exists as two conformers of approximately equal intensity, neither having a plane of symmetry. The presence of two conformers is supported by a <sup>1</sup>H-<sup>13</sup>C NMR heteronuclear single quantum coherence (HSQC) experiment where 12 cross peaks were revealed at -30°C for the CHBr moieties (Fig. 2).

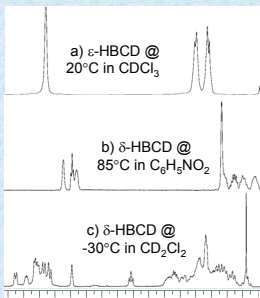


Figure 1. <sup>1</sup>H NMR spectra of  $\delta$ - and  $\epsilon$ -HBCD

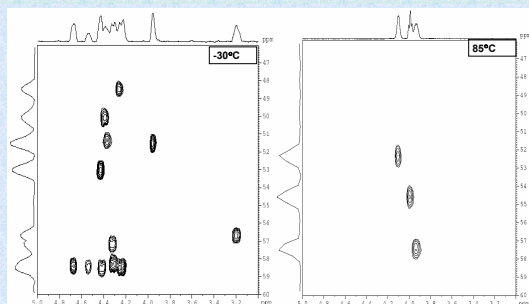


Figure 2. 2D HSQC NMR experiments at -30°C and 85°C for  $\delta$ -HBCD (showing only the NMR region corresponding to the CHBr moiety)

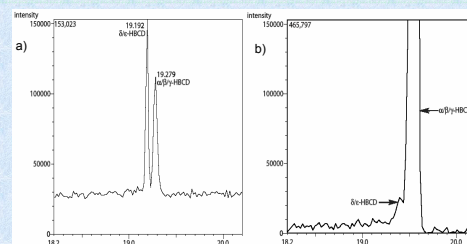


Figure 3. GC chromatogram after injection of a) equi-molar amounts of  $\epsilon$ - and  $\gamma$ -HBCD, b) the technical HBCD mixture

## Results and Discussions (cont')

### GC/MS and LC/MS analysis of **1** and **2**

- GC/MS analysis of a mixture of **1** and **2** gives a single peak indicating that these isomers interconvert similar to the  $\alpha$ -,  $\beta$ -,  $\gamma$ -HBCD.
- GC/MS analysis of **2** and  $\gamma$ -HBCD gives two signals indicating that there is no interconversion between the two HBCD groups (see Fig. 3a).
- LC/MS analysis can separate all five HBCD isomers ( $\alpha$ -,  $\beta$ -,  $\gamma$ -,  $\delta$ - and  $\epsilon$ -HBCD) on a C18 column (see fig. 4).
- The response factors for the molecular ion clusters can vary significantly for the 5 HBCD isomers.

### Analysis of a technical HBCD mixture<sup>4</sup>

- GC/MS analysis shows a small peak attributable to the  $\delta/\epsilon$ -HBCD pair of isomers (see Fig. 3b).
- LC/MS analysis shows small signals arising from isomers with the same retention times of those observed for  $\delta$ - and  $\epsilon$ -HBCD (see Fig. 5).
- The LC chromatogram also reveals the presence of at least three other minor HBCD isomers in this particular sample.
- The LC elution order of  $\delta$ - and  $\epsilon$ -HBCD relative to  $\alpha/\beta/\gamma$ -HBCD is very similar to that of unknown HBCD signals observed in previous studies.<sup>1</sup>

## Conclusions

- Two minor HBCD components ( $\delta$ - and  $\epsilon$ -isomers) previously found in technical grade HBCD have been identified as having structures **1** and **2**.
- 1** and **2** originate from the bromination of residual amounts of ttt-CDT present in technical ctt-CDT.

## References

- Heeb NV, Schweizer WB, Kohler M, Gerecke AC. 2005. Chemosphere, **61**, 65-73.
- Dodder NG, Peck AM, Kucklick JR, Sander LC. 2006. J. Chromatogr. A, **1135**, 36-42.
- Janák K, Nováci A, Voorspoels S, Becher G. 2005. Environ. Sci. Tech. **39**, 1987-1994.
- Results shown here are for the technical HBCD mixture purchased from TCI (lot#H544). Similar results were also obtained for technical HBCD obtained from Acros (lot#A0007134) and 3N International (lot#3N-06303).

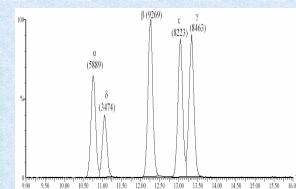


Figure 4. LC chromatogram after injection of equi-molar amounts of the 5 HBCD isomers (value in parenthesis represents approximate integration)

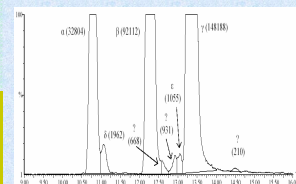


Figure 5. LC chromatogram of the technical HBCD mixture (value in parenthesis represents approximate integration)