

Hexabromocyclododecane **CHALLENGES Scientists** *and* **Regulators**

With 16 potential isomers, this brominated flame retardant is an analytical challenge for environmental scientists and a conundrum for regulators.

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Brominated flame retardants (BFRs) reduce the flammability of a broad range of consumer products. The annual global demand for these high-production-volume chemicals rose to 204,000 tonnes (t) in 2001 (1). Hexabromocyclododecane (HBCD), a technical mixture of several isomers, is primarily used in extruded and expanded polystyrene for thermal insulation in buildings. World production of HBCD in 2001 totalled 16,700 t.

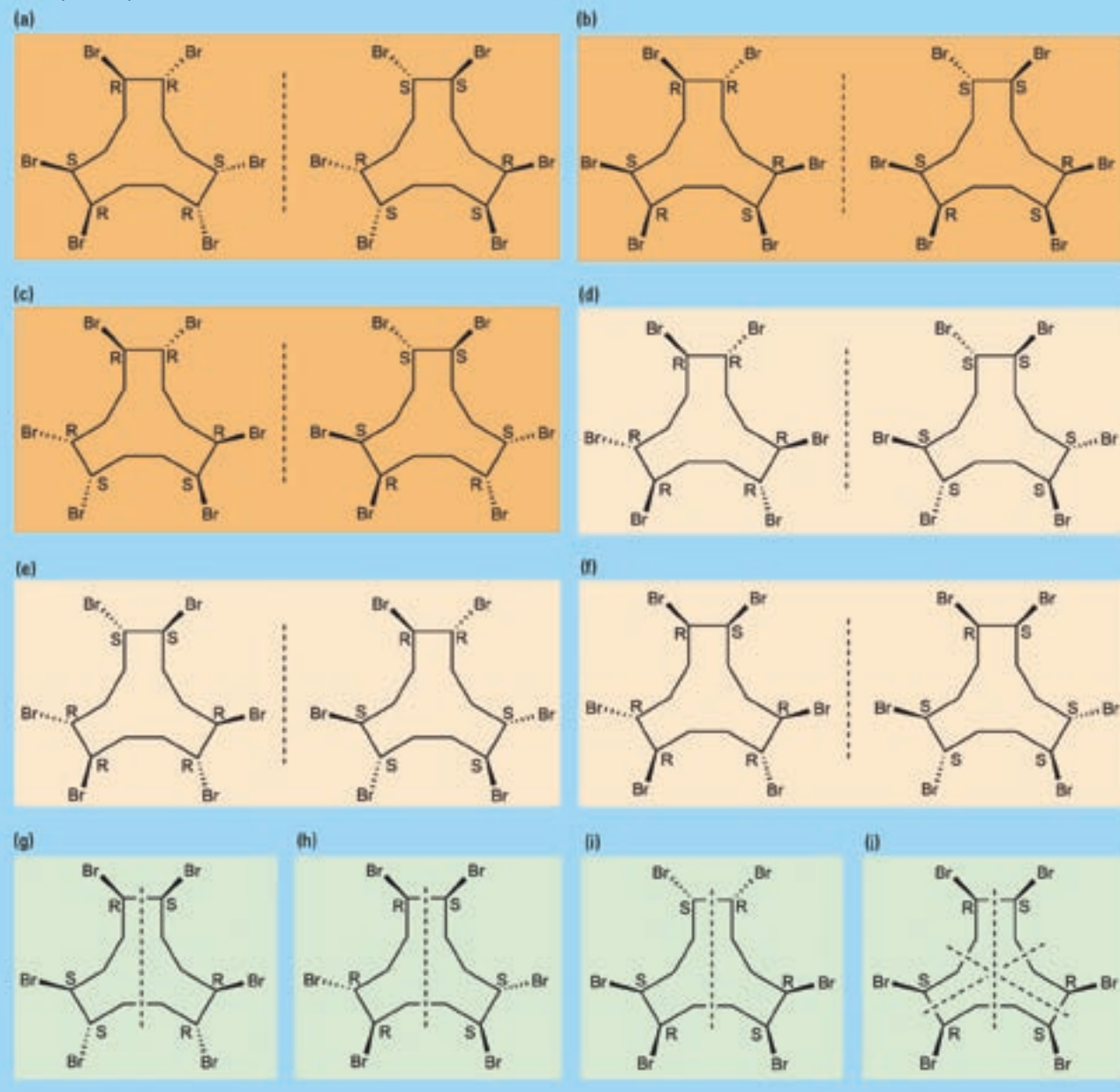
Other common BFRs include tetrabromobisphenol-A (TBBP-A) and decabromodiphenyl ether (DecaBDE). Their annual world market demands are 119,700 and 56,100 t, respectively (2). TBBP-A is used primarily in electronic circuit boards and is chemically bound within the resin. In contrast, DecaBDE and HBCD are simply mixed with the material that is to be flame-retarded. Therefore, these compounds are more likely to leach out during both product use and disposal.

In the mid- to late 1990s, HBCD was first detected in ambient air, sewage sludge, and river sediments in Sweden (3–5). Subsequent studies in many coun-

FIGURE 1

Possible HBCD stereoisomers

Bromination of cyclododeca-1,5,9-trienes theoretically results in 16 stereoisomers—6 enantiomeric pairs (a–f) and 4 meso forms (g–j). The first three pairs of enantiomers (a–c) are assigned as the α -, β -, and γ -HBCD stereoisomers, respectively, which dominate in technical products. So far, structures (d–f) have not been found, whereas two meso forms, whose stereochemistries are not yet assigned, have been detected in a technical product. This graphical representation has been chosen to display all relevant symmetry elements.



tries around the world have confirmed the widespread distribution of HBCD in the environment. As a result, HBCD is currently undergoing a risk assessment within the EU. The main issues outstanding within this assessment are the biodegradability of HBCD and its toxicity in water and sediment.

HBCD's complex stereochemistry

Technical-grade HBCD mixtures are obtained via bromination of cyclododeca-1,5,9-triene (CDT) isomers. Depending on the purity of the starting mate-

rial and the conditions of the industrial processes, a range of technical products with various isomer compositions and melting points are formed.

Figure 1 represents all possible HBCD stereoisomers. Bromination of CDT results in six stereocenters at positions 1, 2, 5, 6, 9, and 10. From all 4 possible CDT isomers, 16 HBCD stereoisomers may be formed, including 6 diastereomeric pairs of enantiomers and 4 meso forms. As a consequence, 10 diastereomers are distinguishable in an achiral environment and 16 stereoisomers in a chiral system.

Structural elucidation of stereoisomers. To date, only three HBCD isomers—labeled α -, β -, and γ -HBCD—have been characterized in technical mixtures (6; Figure 2a), and several contradictory chemical structures have been published. However, eight stereoisomers have been isolated from a low-melting-point technical HBCD mixture (7). The unambiguous identification relied on chromatographic retention, mass spectrometry (MS), X-ray crystal structure analysis, and optical rotation measurements. Five individual HBCD diastereomers—named α -, β -, γ -, δ -, and ϵ -HBCD—were isolated via normal- and reversed-phase (RP) liquid chromatography (LC). The α -, β -, and γ -HBCD diastereomers could each be further resolved into two peaks on a chiral, permethylated β -cyclodextrin stationary phase, whereas δ - and ϵ -HBCD both eluted as single peaks from the chiral column (7).

Optical rotation measurements revealed the presence of three pairs of enantiomers: (–) α - and (–) β -HBCD eluting before the (+) α - and (+) β -HBCD enantiomers, and (+) γ -HBCD eluting ahead of (–) γ -HBCD (Figure 2b). No optical rotation was detected for δ - and ϵ -HBCD. Therefore, these two stereoisomers were tentatively assigned as meso forms. In one low-melting-point technical-product investigation, (+/–) γ -HBCD was the most abundant diastereomer (81.6%). Lower contributions to the total HBCD content of 11.8, 5.8, 0.5, and 0.3% were found for (+/–) α -, (+/–) β -, δ -, and ϵ -HBCD, respectively.

Structural analogy to HCH. The insecticide hexachlorocyclohexane (HCH) has become a textbook example of the relevance of stereospecific processes in the environment. Stereoselective partitioning of HCH in different environmental matrices and relative enrichment of certain stereoisomers during long-range transport have been studied via separation of diastereomers and enantiomers (8, 9). The distribution of HCH stereoisomers in biota is clearly different than in technical mixtures (10). For example, (+/–) α -HCH are the major stereoisomers in technical HCH, but β -HCH is the most abundant diastereomer in biota (11). Furthermore, enantioselective processes must be involved because enantiomeric ratios of (–) and (+) α -HCH differ in biological samples (9, 11, 12). These findings clearly indicate the differing environmental fates of individual HCH stereoisomers. Nowadays, HCH stereoisomers are ubiquitous in the environment and recognized as persistent and bioaccumulating compounds.

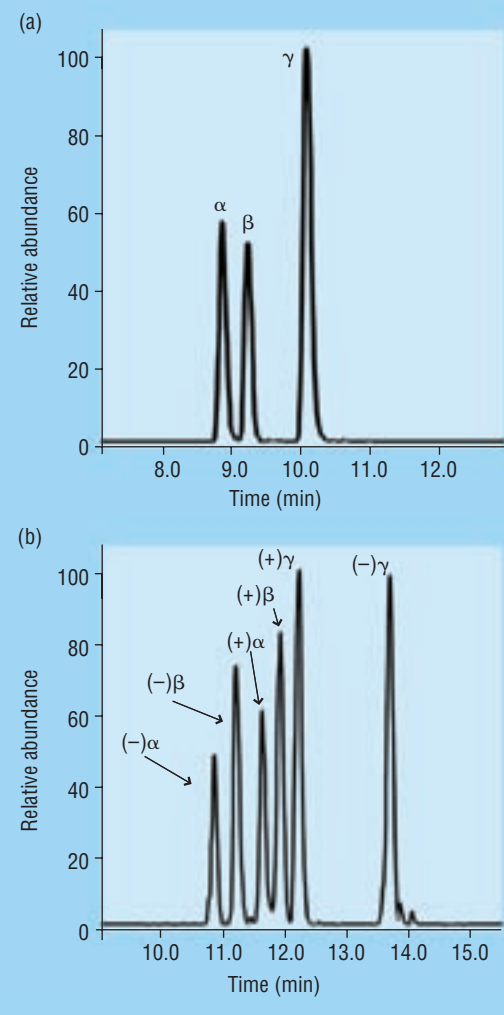
HCH and HBCD have clear parallels. Both have been produced in quantities on the order of 10,000 t per year, and, at first glance, the stereochemistry of HBCD seems to be similar to that of the 1,2,3,4,5,6-HCHs. In both cases, six stereocenters are formed upon complete halogenation. Although 16 stereoisomers are possible for HBCD, only 9 stereoisomers, 7 meso forms, and 1 pair of enantiomers are expected for HCH. Crystal structure analysis revealed that α -HCH is the optically active form and hence can be resolved as two enantiomers, whereas all other stereoisomers are meso forms.

Implications of stereochemistry. It thus seems

FIGURE 2

Separation of α -, β -, and γ -isomers

These HPLC/MS/MS chromatograms show the major hexabromocyclododecane diastereomers on (a) a C_{18} reversed-phase column and (b) a chiral permethylated β -cyclodextrin column.



likely that HBCD, just like HCH, undergoes diastereo- and enantioselective processes in the environment, which induce relative enrichment of different stereoisomers. The latest data confirm this hypothesis. Variations in solubility and partitioning behavior, as well as uptake and metabolism of individual diastereomers, are thought to explain the enrichment of (+/–) α -HBCD in aquatic organisms. As observed with HCH, enantiospecific interaction with biota, different metabolic pathways, and variable biodegradation rates may alter the enantiomeric ratios of (+/–) α -, (+/–) β -, and (+/–) γ -HBCD (13).

Analyzing HBCD

LC/MS/MS is the preferred analytical technique, because it allows both diastereo- and enantioselective determination of HBCD in environmental samples. Gas chromatography (GC) or high-performance LC (HPLC) MS are commonly used for

the quantitative determination of HBCD. However, both of these well-established methods have limitations.

As with many other halogenated persistent organic pollutants (POPs), HBCD has been analyzed via GC/MS, with detection in both the positive and negative ion modes. Because of the higher sensitivity, the mass spectrometer is usually operated in the electron-capture negative-ion (ECNI) mode, with only the bromide ion typically monitored during chromatographic runs. However, larger fragment ions, which are necessary for structural confirmation of HBCD, do not form in ECNI mode.

GC separation has its own serious limitations. It is well known that HBCD diastereomers interconvert at temperatures $>160\text{ }^{\circ}\text{C}$ (14). Because of this thermal isomerization and the fact that HBCD elutes from the GC column at temperatures $>160\text{ }^{\circ}\text{C}$, a broad, unresolved peak is observed (15). Therefore, the total amount of HBCD can be determined by GC but not the quantities of each of the isomers. Moreover, pure HBCD undergoes decomposition by elimination of hydrogen bromide at temperatures $>240\text{ }^{\circ}\text{C}$ (16, 17). Not surprisingly, partial breakdown (15) and even complete loss of HBCD have been reported in GC systems. The thermal exposure of HBCD must therefore be minimized during analysis. Cold on-column injection, short GC columns, thin-film stationary phases, and high flow rates are several measures that can minimize the risk of thermal degradation and reduce the elution temperature.

The brominated compounds that have been used as internal standards, such as brominated diphenyl ethers (BDEs) or brominated biphenyls, have a better thermal stability and therefore cannot be used to compensate for the breakdown of HBCD during GC separation. Furthermore, because isotopically labeled HBCD standards cannot be used when only the bromide ion is monitored, quantifying HBCD by GC/ECNIMS is problematic.

In contrast, RPLC easily separates HBCD isomers (Figure 2a). HPLC, coupled to electrospray ionization (ESI) or atmospheric-pressure chemical ionization MS, is a versatile tool for the isomer-specific determination of HBCD in environmental samples. However, the sensitivity of LC/MS is lower than that of GC/ECNIMS, so HBCD could only be detected in environmental samples with relatively high contamination levels. However, a recently described sensitive and selective method that uses LC/ESI-MS/MS and single reaction monitoring for the transition $[\text{M}-\text{H}]^{-}$ (m/z 640.6) \rightarrow Br^{-} (m/z 79 and 81) yielded a limit of detection (LOD) of 4–6 pg γ -HBCD for a standard solution injected on-column (18). Janák et al. further optimized both the LC and MS conditions to separate the diastereomers and recorded a LOD of 0.5 pg for γ -HBCD in standard solutions and 5–10 pg for γ -HBCD in fish extracts injected on-column (13).

HPLC with chiral, permethylated β -cyclodextrin columns has been successfully used to separate the enantiomers of the (+/-) α -, (+/-) β -, and (+/-) γ -HBCD diastereomers (7, 13). With chiral LC/MS/

MS, Janák et al. fully separated the three pairs of enantiomers of HBCD in a single analysis, with LODs for the different isomers between 10 and 20 pg injected on-column (13; Figure 2b). However, matrix components coeluting with the analytes may lead to serious ion suppression of the primary ion $[\text{M}-\text{H}]^{-}$, resulting in much lower sensitivity. This problem might be avoided with thorough sample cleanup to remove interfering components before HPLC analysis. Furthermore, using isotopically labeled internal standards is essential to compensate for potential variations in sensitivity during and between sample runs. Both ^{13}C -labeled and ^2H -labeled standards of α -, β -, and γ -HBCD are now commercially available. LC/MS results obtained with other internal standards must be regarded as questionable.

In the environment

HBCD has been detected in practically all environmental media and is now considered to be a ubiquitous contaminant. The flame retardant has been detected in both urban and rural air across Sweden and at very remote sites in northern Sweden and Finland, suggesting that it undergoes long-range atmospheric transport (19, 20). This has been confirmed by the detection of HBCD, after atmospheric transport from Western Europe and Eastern North America, in polar bears from Greenland and Svalbard in the Arctic Ocean (20).

The widespread occurrence of HBCD in sewage sludge is a result of diffuse leaching from flame-retarded products into wastewater streams (21). Applying these sludges to agricultural or other land may redistribute the contained HBCD to the soil-sediment compartment and further into aquatic or terrestrial food chains, as demonstrated by BDEs (21).

HBCD has been found in river sediments downstream of urban centers or known industrial sources and in marine sediments at substantially higher concentrations than BDEs (3, 22–24). HBCD has also been detected in both freshwater and marine biota (15, 23, 25). Fish that lived downstream from an HBCD manufacturing plant had very high concentrations of HBCD ($>10\text{ mg/kg}$ wet weight) (23). Food-chain studies have shown that HBCD is bioaccumulative and can be transferred from sediment via invertebrates and predatory fish to fish-eating top predators, such as birds and seals (23, 24). A Swedish study of the changes over time in HBCD concentrations in archived Baltic Sea guillemot eggs indicated an increase in concentrations since 1969. However, during the past decade, concentrations in those eggs have stabilized (25).

The major intake of HBCD for humans is from food and indoor air or dust. According to the few data available on levels of HBCD in food, fish appear to be a major source (19). Furthermore, HBCD has been found in house dust at very high concentrations (26–28), an indication that indoor exposure through inhalation or ingestion is highly likely and could contribute significantly to human exposure. However, the levels of HBCD observed so far in hu-

man milk (29) and blood (30) are low and in the same range as those measured for hexa-BDE congeners.

A few studies have reported on the environmental levels of individual HBCD diastereomers. The diastereomeric profile of HBCD in most sediment samples is similar to that of commercial formulations: γ -HBCD is the most abundant isomer (23, 31). However, in some locations, a considerable contribution from α -HBCD has been observed (22, 23, 32). Temperatures reach >160 °C during the production of certain polymers, and this may lead to the thermal conversion of γ - to α -HBCD (22, 23). In those cases, the HBCD source is likely the production of heat-treated polymer materials or textiles and not an HBCD technical product itself.

In contrast to sediments, the α -isomer seems to dominate the HBCD profile in the vast majority of biota (24, 31–34). Several factors may cause this change. First, the elution order in RPLC indicates that α -HBCD is more hydrophilic than β - or γ -HBCD; this is in accordance with the water solubility determined for the individual HBCD diastereomers (35). Second, it has been shown that liver microsomes from marine mammals metabolize β - and γ -HBCD more rapidly than α -HBCD and thereby form hydroxy-metabolites (34). Finally, evidence for the bio-isomerization of HBCD diastereomers in fish has been recently reported (36). Significant concentrations of the other isomers were detected in fish experimentally exposed only to α - or γ -HBCD. In both cases, the β -isomer was formed least often. All these observations are in accordance with the HBCD profile generally found in fish, which shows a dominance of the α - over the γ - isomer, and little or no β -isomer. The differing isomer profiles are shown in Figure 3. To date, the γ - and ε -HBCD isomers have not been found in environmental samples.

HBCD enantiomers can exhibit different biotransformation and biological activities, so researchers must also evaluate this possibility. Janák et al. have already used chiral LC/MS/MS to demonstrate the selective accumulation of the HBCD enantiomers in marine fish (13).

Toxicity

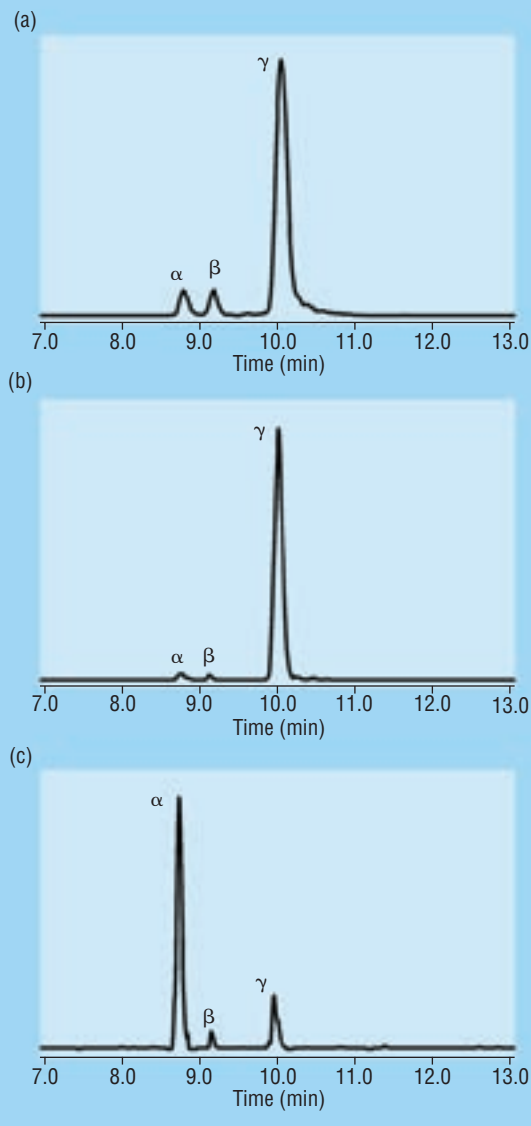
The toxicological database for HBCD lacks relevant, high-quality studies (37). The direct acute and chronic toxicity of HBCD appears to be low, but sublethal effects can't be ruled out. HBCD has an antagonistic effect on detoxification enzymes and thus may also increase the toxicity of other compounds (38). It has also been suggested that HBCD may induce cancer by a nonmutagenic mechanism (38, 39) and may disrupt the thyroid hormone system (37, 40, 41).

The endocrine-disrupting activity of HBCD is currently under investigation within the EU-funded project Flame Retardants Integrated Risk Assessment for Endocrine Effects (FIRE; 42). Neonatal exposure to HBCD can induce developmental neurotoxic effects, such as aberrations in spontaneous behavior, learning, and memory function (43, 44). HBCD can also alter the normal uptake of the neu-

FIGURE 3

α -HBCD isomer dominates biota

These chromatograms were obtained by HPLC/MS/MS for (a) a technical mixture, (b) a sediment sample from the Western Scheldt estuary in The Netherlands, and (c) plaice (fish) liver from the same area.



rotransmitters in rat brains (45). Last year, Birnbaum and Staskal concluded that further studies are needed on developmental effects, endocrine disruption, and longer-term effects such as carcinogenesis (46). To date, no information exists on the relative toxicity of the different HBCD diastereomers and enantiomers. Also, until very recently, toxicological testing has been conducted solely with technical HBCD products rich in γ -HBCD, whereas internal exposure may be dominated by other isomers.

Regulation and risk assessment

Different countries have various strategies and legal frameworks to assess the safety of HBCD in the environment. Within the EU, HBCD is covered by

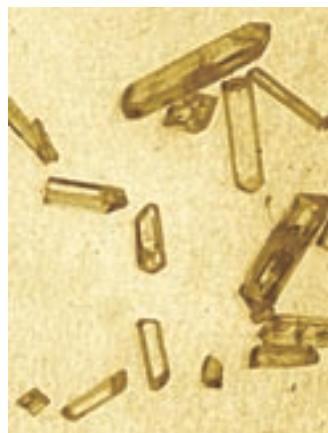
the Council Regulation 793/93 on the Evaluation and Control of the Risks of Existing Substances. The EU risk assessment for HBCD began in 1997, with Sweden as the lead country. A draft risk assessment report is currently under discussion within the EU. Additional testing of the degradability of the individual isomers will be necessary before a definitive conclusion on the persistence of HBCD can be drawn. The bromine industry has agreed to conduct further studies on degradation products of HBCD.

According to the European Brominated Flame Retardant Industry Panel, data from new toxicity, fate, and emission studies have been submitted recently. Bert-Ove Lund, head of the New and Existing Substances Unit at the Swedish Chemicals Inspectorate, anticipates that the EU risk assessment process for HBCD will be completed during 2005. In its current draft version, the HBCD risk assessment states that further information and testing are required; a need also exists to limit the risks to human health and the environment.

The U.S. EPA addresses flame retardants under the Toxic Substances Control Act of 1976. EPA is currently developing a rule to complement a national flammability standard for residential upholstered furniture, which is under consideration by the Consumer Product Safety Commission (CPSC). This rule would require notification and review of 16 flame-retardant chemicals or categories of chemicals, including HBCD, which have been identified by CPSC and industry as likely to be used to flame-retard fabrics on furniture. According to Kenneth Moss from EPA's Office of Pollution Prevention and Toxics, this is the only formal mention of HBCD in any regulatory context in the United States.

Canadian regulators identified HBCD and four halogenated cyclohexanes for assessment during the next few years. In Japan, manufactured and imported amounts of HBCD must be reported to the government. Further steps include preliminary toxicity evaluation and, if necessary, guidance and advice for risk reduction, release minimization measures, and long-term toxicity investigation. In Australia, the National Industrial Chemicals Notification and Assessment Scheme is currently updating information on use, importation, and production of BFRs, including HBCD, to determine the need for a full risk assessment and further regulatory activities.

Environmental monitoring and quantification of HBCD emissions under the voluntary emissions reduction program of the Bromine Science and Environmental Forum have shown that emission control from both HBCD production and usage is feasible for even small- and medium-sized enterprises, according to the Organisation for Economic Co-operation and Development. The Commission for the Protection of the Marine Envi-



ronment of the North-East Atlantic has included HBCD on its list of chemicals for priority action to protect the marine environment. And as yet, no official position exists on HBCD as a POP candidate, according to Heidelore Fiedler, a scientific affairs officer with the UN Environment Programme. At present, HBCD is included in neither the list of 27 chemicals under the Rotterdam Convention on Prior Informed Consent (PIC) nor the initial list of 12 POPs named within the Stockholm Convention on POPs. But both conventions have a

mechanism to add new chemicals: New PIC chemicals or new POPs can be added following proposals from individual member countries, evaluation in the review committee of the respective convention, and acceptance by the Conference of the Parties.

Challenges ahead

The risk assessment of HBCD is not yet complete. Because of HBCD's complex stereochemistry, isomer-specific data are needed to elucidate its sources, distribution, and fate for an assessment of the risks related to this bioaccumulative compound. We urge all environmental scientists to use LC/MS in all applicable studies and adopt a diastereomer- and enantiomer-specific approach to HBCD analysis. Improving the quality and breadth of available analytical data will help regulators and risk assessors evaluate the risks associated with its continued use.

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References

- (1) Bromine Science and Environmental Forum Frequently Asked Questions, www.bsef-site.com/bromine/faq/index.php.
- (2) Bromine Science and Environmental Forum, www.bsef.com.
- (3) Sellström, U.; et al. Polybrominated Diphenyl Ethers and Hexabromocyclododecane in Sediment and Fish from a Swedish River. *Environ. Toxicol. Chem.* **1998**, *17*, 1065-1072.
- (4) Sellström, U.; et al. Brominated Flame Retardants in Sediments from European Estuaries, the Baltic Sea and in Sewage Sludge. *Organohalogen Compd.* **1999**, *40*, 383-386.
- (5) de Wit, C. Brominated Flame Retardants; Report No. 5065; Swedish Environmental Protection Agency: Stockholm, Sweden, 2000.
- (6) Becher, G. The Stereochemistry of 1,2,5,6,9,10-Hexabro-

- mocyclododecane and Its Graphic Representation. *Chemosphere* **2005**, *58*, 989–991.
- (7) Heeb, N. V.; et al. 1,2,5,6,9,10-Hexabromocyclododecanes—A Class of Compounds with a Complex Stereochemistry. *Chemosphere* **2005**, in press.
 - (8) Kallenborn, R.; et al. Ambient Air Levels and Atmospheric Long-Range Transport of Persistent Organochlorines to Signy Island, Antarctica. *Sci. Total Environ.* **1998**, *220*, 167–180.
 - (9) Wiberg, K.; et al. The Enantioselective Bioaccumulation of Chiral Chlordane and α -HCH Contaminants in the Polar Bear Food Chain. *Environ. Sci. Technol.* **2000**, *34*, 2668–2674.
 - (10) Willett, K. L.; Ulrich, E. M.; Hites, R. A. Differential Toxicity and Environmental Fates of Hexachlorocyclohexane Isomers. *Environ. Sci. Technol.* **1998**, *32*, 2197–2207.
 - (11) Covaci, A.; Gheorghe, A.; Schepens, P. Distribution of Organochlorine Pesticides, Polychlorinated Biphenyls and α -HCH Enantiomers in Pork Tissues. *Chemosphere* **2004**, *56*, 757–766.
 - (12) Buser, H.-R.; Müller, M. D. Isomer and Enantioselective Degradation of Hexachlorocyclohexane Isomers in Sewage Sludge under Anaerobic Conditions. *Environ. Sci. Technol.* **1995**, *29*, 664–672.
 - (13) Janák, K.; et al. Hexabromocyclododecane (HBCD) in Marine Species from the Western Scheldt Estuary: Diastereomer- and Enantiomer-Specific Accumulation. *Environ. Sci. Technol.* **2005**, *39*, 1987–1994.
 - (14) Peled, M.; Scharia, R.; Sondack, D. Thermal Rearrangement of Hexabromocyclododecane (HBCD). In *Advances in Organobromine Chemistry II*; Desmurs, J.-R., Gérard, B., Goldstein, M. J., Eds.; Elsevier: Amsterdam, The Netherlands, 1995; pp 92–99.
 - (15) Eljarrat, E.; et al. Occurrence and Bioavailability of Polybrominated Diphenyl Ethers and Hexabromocyclododecane in Sediment and Fish from the Cinca River, a Tributary of the Ebro River (Spain). *Environ. Sci. Technol.* **2004**, *38*, 2603–2608.
 - (16) Barontini, F.; Cozzani, V.; Petarca, L. Thermal Stability and Decomposition Products of HBCD. *Ind. Eng. Chem. Res.* **2001**, *40*, 3270–3280.
 - (17) Larsen, E. R.; Ecker, E. L. Thermal Stability of Fire Retardants: I, Hexabromocyclododecane (HBCD). *J. Fire Sci.* **1986**, *4*, 261–275.
 - (18) Budakowski, W.; Tomy, G. Congener-Specific Analysis of Hexabromocyclododecane by High-Performance Liquid Chromatography/Electrospray Tandem Mass Spectrometry. *Rapid Commun. Mass Spectrom.* **2003**, *17*, 1399–1404.
 - (19) Remberger, M.; et al. The Environmental Occurrence of Hexabromocyclododecane in Sweden. *Chemosphere* **2004**, *54*, 9–21.
 - (20) de Wit, C.; Alae, M.; Muir, D. Brominated Flame Retardants in the Arctic—An Overview of Spatial and Temporal Trends. *Organohalogen Compd.* **2004**, *66*, 3811–3816.
 - (21) Law, R. J.; et al. Levels and Trends of Brominated Flame Retardants in the European Environment. *Chemosphere* **2005**, in press.
 - (22) Marvin, C. H.; et al. Distribution of Hexabromocyclododecane in Detroit River Suspended Sediments. *Chemosphere* **2005**, in press.
 - (23) Morris, S.; et al. Distribution and Fate of HBCD and TBBPA Flame Retardants in North Sea Estuaries and Aquatic Food Webs. *Environ. Sci. Technol.* **2004**, *38*, 5497–5504.
 - (24) Leonards, P.; et al. Biotransformation of Polybrominated Diphenyl Ethers and Hexabromocyclododecane in Two Dutch Food Chains. In *Proceedings of the Third International Workshop on Brominated Flame Retardants*, BFR 2004, Toronto, Canada, 2004; pp 283–286, www.bfr2004.com.
 - (25) Sellström, U.; et al. Temporal Trend Studies on Tetra- and Pentabrominated Diphenyl Ethers and Hexabromocyclododecane in Guillemot Egg from the Baltic Sea. *Environ. Sci. Technol.* **2003**, *37*, 5496–5501.
 - (26) Leonards, P. E. G.; et al. Brominated Flame Retardants in Office Dust Samples. In *Proceedings of the Second International Workshop on Brominated Flame Retardants*, BFR 2001, Stockholm, Sweden, 2001; pp 299–302, www.bfr2004.com (see BFR 2001).
 - (27) Knoth, W.; et al. Brominated Diphenyl Ethers in Indoor Dust. *Organohalogen Compd.* **2003**, *61*, 207–210.
 - (28) Stapleton, H. M.; et al. Measurement of the Flame Retardants Polybrominated Diphenyl Ethers (PBDEs) and Hexabromocyclododecane (HBCDD) in House Dust. *Organohalogen Compd.* **2004**, *66*, 3740–3744.
 - (29) Thomsen, C.; et al. Brominated Flame Retardants in Breast Milk from Norway. *Organohalogen Compd.* **2003**, *64*, 33–36.
 - (30) Weiss, J.; et al. PBDE and HBCDD Levels in Blood from Dutch Mothers and Infants. In *Proceedings of the Third International Workshop on Brominated Flame Retardants*, BFR 2004, Toronto, Canada, 2004; pp 71–74, www.bfr2004.com.
 - (31) Allchin, C. R.; Morris, S. Hexabromocyclododecane (HBCD) Diastereoisomers and Brominated Diphenyl Ether Congener (BDE) Residues in Edible Fish from the Rivers Skerne and Tees, U.K. *Organohalogen Compd.* **2003**, *61*, 41–44.
 - (32) Schlabach, M.; Fjeld, E.; Borgen, A. R. Brominated Flame Retardants in Drammen River and the Drammensfjord, Norway. In *Proceedings of the Third International Workshop on Brominated Flame Retardants*, BFR 2004, Toronto, Canada, 2004; pp 147–150, www.bfr2004.com.
 - (33) Gerecke, A. C.; et al. Detection of Alpha-Isomer Dominated HBCD (Hexabromocyclododecane) in Swiss Fish at Levels Comparable to PBDEs (Polybrominated Diphenyl Ethers). *Organohalogen Compd.* **2003**, *61*, 155–158.
 - (34) Zegers, B. N.; et al. Levels of Hexabromocyclododecane in Harbour Porpoises and Common Dolphins from Western European Seas, with Evidence for Stereoisomer-Specific Biotransformation by Cytochrome P450. *Environ. Sci. Technol.* **2005**, *39*, 2095–2100.
 - (35) Hunziker, R. W.; et al. Fate and Effect of Hexabromocyclododecane in the Environment. *Organohalogen Compd.* **2004**, *66*, 2300–2305.
 - (36) Law, K.; et al. Evidence of Bioisomerization of α - and γ -Hexabromocyclododecane (HBCD) Isomers in Fish. In *Proceedings of the Third International Workshop on Brominated Flame Retardants*, BFR 2004, Toronto, Canada, 2004; pp 383–386, www.bfr2004.com.
 - (37) Darnerud, P. O. Toxic Effects of Brominated Flame Retardants in Man and in Wildlife. *Environ. Int.* **2003**, *29*, 841–853.
 - (38) Ronisz, D.; et al. Sublethal Effects of the Flame Retardants Hexabromocyclododecane (HBCDD) and Tetrabromobisphenol A (TBBPA) in Juvenile Rainbow Trout (*Oncorhynchus mykiss*). *Aquat. Toxicol.* **2001**, *69*, 229–245.
 - (39) Helleday, T.; et al. Brominated Flame Retardants Induce Intragenic Recombination in Mammalian Cells. *Mutat. Res.* **1999**, *439*, 137–147.
 - (40) Hall, A. J.; Kalantzi, O. I.; Thomas, G. O. Polybrominated Diphenyl Ethers (PBDEs) in Grey Seals During Their First Year of Life—Are They Thyroid Hormone Endocrine Disruptors? *Environ. Pollut.* **2003**, *126*, 29–37.
 - (41) Sakai, H.; et al. Effects of Brominated Flame Retardants on Transcriptional Activation Mediated by Thyroid Hormone Receptor. *Organohalogen Compd.* **2003**, *61*, 215–218.
 - (42) Flame Retardants Integrated Risk Assessment for Endocrine Effects, www.rivm.nl/fire.
 - (43) Eriksson P.; et al. A Comparison on Developmental Neurotoxic Effects of Hexabromocyclododecane, 2,2',4,4',5,5'-Hexabromodiphenylether (PBDE 153) and 2,2',4,4',5,5'-Hexachlorobiphenyl (PCB 153). *Organohalogen Compd.* **2002**, *57*, 389–392.
 - (44) Eriksson, P.; et al. Comparative Developmental Neurotoxicity of Flame Retardants, Polybrominated Flame Retardants and Organophosphorus Compounds, in Mice. *Organohalogen Compd.* **2004**, *66*, 3163–3165.
 - (45) Mariussen, E.; Fonnum, F. The Effect of Brominated Flame Retardants on Neurotransmitter Uptake into Rat Brain Synaptosomes and Vesicles. *Neurochem. Int.* **2003**, *43*, 533–542.
 - (46) Birnbaum, L. S.; Staskal, D. F. Brominated Flame Retardants: Cause for Concern? *Environ. Health Perspect.* **2004**, *112*, 9–17.