HEXABROMOCYCLODODECANES IN INDOOR DUST FROM CANADA, THE UNITED KINGDOM AND THE UNITED STATES

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Abstract
Hexabromocyclododecane (HBCD) diastereomers were analyzed in house dust samples from Birmingham, UK (n=31); Amarillo/Austin, USA (n=13); and Toronto, Canada (n=8). Six samples of dust from UK offices were also studied. A ΣHBCD concentration of 110,000 ng/g was recorded in one of the UK house dust samples. Concentrations of ΣHBCD in house dust samples from each country were statistically indistinguishable, and concentrations in UK office samples fell within the range observed for UK homes. Ingestion of household dust is shown to constitute an important pathway of exposure to the UK population. The concentrations of α-HBCD in dust samples were generally higher than those present in the commercial formulations and it was the dominant diastereomer in a small number of samples. This is consistent with the thermal instability of HBCD, where at the temperatures used to incorporate the commercial formulations into treated goods (above 160 °C), γ- HBCD is partially converted to the α-isomer[1]. This – together with the fact that cytochrome P450 preferentially metabolizes the γ- and β-diastereomers but not the α-diastereomer[2] - provides a likely explanation for the observed dominance of the α-diastereomer in human tissues, compared to the dominance of the γ-diastereomer in the commercial formulations.

Introduction
Hexabromocyclododecane (HBCD) is a brominated flame retardant used widely as an additive to expanded and extruded polystyrene foams for thermal insulation and to a lesser extent for back-coating of fabrics for furniture. In 2001, the world market demand for HBCD was 16,700 tons of which about 9,500 tons were consumed in Europe[3]. It is an aliphatic, brominated cyclic alkane produced by bromination of cyclododecatri-1,5,9-ene[4]. The commercial mixtures consist mainly of the α-, β-, and γ-diastereomer with the latter predominant. HBCD is characterised by having a low vapour pressure, very low water solubility and a log K_{OW} value of 5.6. It can therefore bioaccumulate in fatty tissues. It is also persistent, with estimated half-lives of 3 days in air and 2–25 days in water, that bestow potential for long-range transport[5]. HBCD can enter the environment by different pathways including emission during its production or manufacture of the flame-retarded materials and then final products, leaching from products or following product disposal. It was found that oral exposure to HBCD induces hepatic cytochrome P450 enzymes in rats and can alter the normal uptake of neurotransmitters in rat brain. There are other indications that it can disrupt the thyroid hormone system and induce cancer through a non-mutagenic mechanism in humans[5]. HBCD has been recognised by the U.K. Chemical Stakeholders Forum as a persistent, bioaccumulative, and toxic chemical and is included on the OSPAR list of chemicals for priority action. While currently no specific regulatory actions are taken in the United States, HBCD has been identified for risk assessment in Canada, Australia, and Japan. Further regulatory/assessment activities in these countries will take place over the next few years[6].

Given the current dearth of information concerning human exposure to HBCD, coupled with mounting evidence of the importance of the ingestion of indoor dust as a human exposure pathway to another class of brominated flame retardants (PBDEs), this study measures concentrations of the three principal diastereomers in indoor dust. These concentrations are used to provide a preliminary assessment of human exposure to HBCD via dust ingestion. Furthermore, given the observed order-of-magnitude differences between North American and European exposure via this pathway to Σtri-hexa-BDEs[7], dust samples are examined from Canada, the UK, and the US.

Materials and Methods
Dust samples were collected through a standardized procedure using a vacuum cleaner. Further details are

provided elsewhere[7]. The samples were sieved through a 500 μm mesh size sieve, accurately weighed then extracted using pressurized fluid extraction (Dionex, ASE 300). Dust samples (1 g) were loaded into pre-cleaned 66 mL cells containing 1.5 g Florisil and Hydromatrix (Varian Inc.) to fill the void volume of the cells, spiked with 10 ng of each of ^{13}C-labelled α-, β, and γ-HBCD as internal standards and extracted under the following conditions:

* Solvent: Hexane/CH_2Cl_2 (1:1) * Pressure: 1500 psi * Temperature: 90˚C
* Heating time: 6 min * Static time: 5 min * Flush Volume: 50%
* Purge time: 100 secs * Static Cycles: 3

The crude extracts were concentrated to 0.5 mL using a Zymark Turbovap® II then washed with 98% sulfuric acid. The hexane layer was transferred onto a Florisil column topped with sodium sulfate and then eluted with 30 mL of hexane/CH_2Cl_2 (1:1). The eluate was concentrated under a gentle stream of N_2, solvent exchanged into methanol and 2 ng of d_{18}-γ-HBCD added to each sample as a recovery determination standard. Separation of α-, β-, and γ- HBCD was achieved using a dual pump Shimadzu LC-20AB prominence liquid chromatograph equipped with SIL-20A autosampler and DGU-20A3 vacuum degasser. A Varian Pursuit XRS3 C_{18} reversed phase analytical column (150 mm x 2 mm i.d., 3 μm particle size) was used. A mobile phase of (a) 1:1 water/methanol with 2 mM ammonium acetate and (b) methanol at a flow rate of 150 μL/min was applied for elution of the target compounds; starting at 50% (b) then increased linearly to 100% (b) over 3 min; this was held for 5 minutes followed by a linear decrease to 65% (b) over 2.5 minutes and held for 3.5 minutes. Mass spectrometric analysis was performed using a Sciex API 2000 triple quadrupole mass spectrometer operated in the ES negative ion mode. Infusion experiments utilized the built-in Harvard syringe pump with a flow rate of 10 μL/min. MS/MS detection operated in the MRM mode was used for quantitative determination of the HBCD isomers based on m/z 640.6→ m/z 79, m/z 652.4→ m/z 79 and m/z 657.7→ m/z 79 for the native, ^{13}C-labelled and d_{18}-labelled diastereomers respectively.

Statistical analysis of the data was conducted using Excel (Microsoft Office for Mac OS X) to generate descriptive statistics, with other statistical procedures conducted using SPSS version 13.0 for Mac OS X.

Results and Discussion

The three diastereomers were baseline separated on the reversed phase C_{18} column with retention times of 9.4, 9.9 and 10.3 minutes for α-, β- and γ- HBCD respectively. Recoveries of the three ^{13}C-HBCD diastereomers ranged from 70 to 93%. The accuracy of the applied analytical method was checked via replicate analysis (n=5) of SRM2585 and excellent results obtained (Table 1).

	Average concentration (ng/g) ± standard deviation (n=5)	
	Measured	**Indicative**
α-HBCD	19±3.6	19±3.7
β-HBCD	4.4±0.4	4.3±1.1
γ-HBCD	125±18	120±22

Table 1: Concentrations of HBCD isomers in SRM2585 compared to the indicative values[8].

All 3 diastereomers of HBCD were detected and quantified in all dust samples (Table 2). Of particular note is the very high concentration of ΣHBCD (110,000 ng/g) detected in one UK house dust sample. The ΣHBCD data in house dust samples were then statistically evaluated for differences in concentrations between the three countries studied. As a first step, the distribution of concentrations within each national dataset was evaluated using both Shapiro-Wilks and Kolmogorov-Smirnov tests. The results – combined with visual inspection of frequency diagrams - revealed the data for both the UK and the US to be log-normally distributed, while that for Canada displayed a normal distribution. Hence, we performed two ANOVA tests: one on log-transformed concentrations and another on non-transformed concentrations. Both analyses suggested no significant differences ($p>0.05$ as measured using Scheffe and Bonferroni indicators) in ΣHBCD concentrations among the three countries, although it cannot be excluded that a larger survey may reveal differences. The limited number of UK office samples studied (n=6) precludes statistical analysis, but concentrations in office samples fall within the range observed in homes.

	UK homes (n=31)				UK offices (n=6)			
	α-HBCD	β-HBCD	γ-HBCD	ΣHBCD	α-HBCD	β-HBCD	γ-HBCD	ΣHBCD
Average	2800	470	2800	6000	250	160	1000	1400
Median	170	66	440	730	100	75	470	650
SD	12000	1500	7800	21000	270	160	1100	1500
Minimum	22	9	70	140	15	11	65	90
Maximum	66000	7800	37000	110000	630	380	2600	3600
Average % of ΣHBCD	32	8	60	-	21	10	70	-
	Canadian homes (n=8)				**US homes (n=13)**			
	α-HBCD	β-HBCD	γ-HBCD	ΣHBCD	α-HBCD	β-HBCD	γ-HBCD	ΣHBCD
Average	340	70	260	670	260	56	490	810
Median	300	72	230	640	80	28	300	390
SD	230	44	160	420	480	82	600	1100
Minimum	25	6	34	64	17	6	79	110
Maximum	670	130	470	1300	1800	300	2000	4000
Average % of ΣHBCD	49	10	41	-	28	7	65	-

Table 2: Summary of concentrations (ng/g) of HBCD diastereomers in indoor dust.

The measured concentrations were used to estimate the exposure of UK adults and toddlers to ΣHBCD via dust ingestion (Table 3). To do so, we have assumed (in the absence of experimental data) 100% absorption of intake, average adult and toddler dust ingestion figures of 20 and 50 mg/day, and high dust ingestion figures for adults and toddlers of 50 and 200 mg/day[9]. It is stressed that the range of exposure estimates via dust ingestion thus derived are only an indication of the likely range within the population. Comparing the obtained estimates via dust ingestion to the UK estimated upper bound dietary intake from the whole diet in 2004 (413 ng ΣHBCD/day for a 70 kg adult, and 240 ng ΣHBCD/day for a 10 kg toddler)[10], it is clear that ingestion of indoor dust represents an important pathway for human exposure to HBCD within the UK.

	Adult				Toddler (6-24 months)			
Intake (ng ΣHBCD/ day)	5th %ile	Median	Average	95th %ile	5th %ile	Median	Average	95th %ile
Canada (mean dust ingestion)	3.0	13	13	24	7.5	32	33	59
Canada (high dust ingestion)	7.5	32	33	59	30	130	130	240
UK (mean dust ingestion)	3.2	15	120	440	8.0	37	300	1100
UK (high dust ingestion)	8.0	37	300	1100	32	150	1200	4400
US (mean dust ingestion)	2.3	7.7	16	60	5.8	19	40	150
US (high dust ingestion)	5.8	19	40	150	23	77	160	600

Table 3: Summary of estimates of exposure (ng/day) of adults and toddlers to ΣHBCD via dust ingestion in Canada, the UK, and the US.

The relative abundances of the three principal HBCD diastereomers varies substantially among dust samples. This may be explained partly by the existence of four different grades of the HBCD commercial formulation, each containing different percentages of the three diastereomers: low melt, medium range, high melt, and thermally stabilized. Which grade is used depends on the product to be treated[11]. However, this cannot account for the fact that in many of the dust samples studied in this project, the percentage of the α-HBCD diastereomer exceeds substantially that found in the commercial mixtures, which are dominated by the γ-diastereomer. This is thought attributable largely to the thermal instability of γ-HBCD;

specifically, above 160°C, γ- HBCD can be converted to the α-isomer (Figure 1). It is thought that the process of incorporating the HBCD commercial formulations into a polymer matrix requiring flame-retarding, requires thermal treatment at or above this temperature, leading to such thermal rearrangement, and associated changes in the diastereomer distribution in the treated product [1,11].

(a) (b)

Figure 1: Chromatograms showing the relative abundance of HBCD isomers in a technical mixture (a) and in a technical mixture after thermal treatment above 160°C (b).

This finding – together with the fact that the cytochrome P450 system readily metabolizes the γ- and β-diastereomers but not the α-diastereomer[2] - may provide an explanation for the observed predominance of the α-diastereomer in human tissue samples.

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