HEXABROMOCYCLODODECANE (HBCD) CONCENTRATIONS FROM AN AUSTRALIAN POPULATION IN 2010/11

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Introduction

Brominated flame retardants including hexabromocyclododecane (HBCD) are described as cost effective and highly efficient ways to reduce flammability and therefore potentially reduce the harm to humans caused by fires. HBCD has been used since the 1960s [1] in products such as upholstered textiles, rigid form plastics and polystyrene foam used in baby car seats [2]. The commercial HBCD product usually contains three steroisomers alpha (α-), beta (β-) and gamma (γ-) at approximately 6%, 8% and 80% [3]. HBCD is an additive flame retardants and can therefore leach or volatilise from products and enter the environment and humans.

In Australia, the importation of HBCD raw product increased by 78% between 1998/99 and 2003/04 [4]. However, there has been a decrease in the import of HBCD over recent years, with approximately 90 tonnes imported in 2006-07 and about 60 tonnes in 2009-10. Import into Australia of HBCD as powder or granules ceased in 2010 [5]. The commercial product HBCD has a high bioaccumulative and biomagnification potential [6] and it is currently under consideration for inclusion in the Stockholm Convention on persistent organic pollutants. Data on human health effects of both HBCD exposure is limited. Eggesbo et al. [7] reported no association between thyroid stimulating hormone and exposure within the exposure level recorded. Dorosh et al. [8] used human breast cancer cell lines and reported that HBCD at higher concentrations displayed oestrogenic properties and therefore has the potential to disrupt the endocrine system.

While extensive studies have been conducted on the human body burden, sources and exposure pathways of polybrominated diphenyl ethers (PBDEs) in Australia [9, 10], data on HBCD is still limited. Due to the longevity of products containing HBCD and changes in usage rates, these products will remain in use for some time with the potential to provide both primary and secondary sources to the environment and humans. An investigation of HBCD in human breast milk from Australia found detectable concentrations of HBCD but variable temporal trends from 1993 – 2009.

The main objective of this study is to provide the first data on HBCD in the Australian population from human blood serum. This will enable comparisons with HBCD concentrations by age and gender. The data are essential for future monitoring of HBCD body burden trends in Australia and to determine if an assessment of sources and exposure pathways is warranted.

Materials and methods

Pooled samples of human blood serum collected in South East Queensland, Australia in 2010/11. The benefits and limitations of sample pooling have been discussed in detail in Heffernan et al. [11]. All samples were obtained in collaboration with Sullivan Nicolaides Pathology (SNP) from de-identified surplus pathology samples and were stratified by age and gender. Age groups were as follows: 0-4; 5 – 15; 16-30, 31-45, 46-60, and > 60 years. For each age group, samples were collected from both genders and in replicate (6 age groups x 2 genders x 2 replicates = 24 pools). Pooled samples were placed into 100 ml solvent rinsed glass bottles with 1

ml of each of the 100 samples pooled to total 2400 samples into 24 pools. Ethics approval for this study was granted by The University of Queensland Medical Research Ethics Committee.

Pooled samples were inverted 20 times before 5 mL was transferred to clean 50 mL Falcon tubes. Samples spiked with 0.5 ng $^{13}C_{12}$-labelled a, b and g –HBCD internal standards. 5 mL (50% concentrated) formic acid was added to samples before vortexing for 1 minute. Samples were extracted based on methods by Roosens et al. [12]. Briefly, they were sonicated for 20 minutes and left at 4 °C overnight. Oasis HLB SPE cartridges (6 mL/500 mg, Waters) were pre-cleaned with 6 mL DCM and 6 mL MeOH and conditioned with 5 mL milliQ water. Samples were loaded onto the cartridges and allowed to absorb to the cartridge gravimetrically. Samples were then dried under vacuum for 10 minutes before being eluted into glass tubes with 12 mL DCM. The extracts were concentrated to <0.25 mL under a gentle stream of nitrogen and reconstituted in around 1 mL hexane and mixed with 1 mL <98% concentrated sulfuric acid and left to separate. The top hexane layer, along with 3 x 1 mL hexane rinses were transferred into clean glass tubes and concentrated to 1 mL. Samples were loaded onto Supelco LC-Si cartridges (3 mL / 500 mg) and fractionated using a method modified from Shi et al. [13]. Cartridges were pre-cleaned with 2 x 3 mL DCM and conditioned with 3 mL hexane. The 1 mL hexane extracts were sonicated for 1 min before being transferred onto the cartridge and absorbed gravimetrically. The first fraction was eluted with 6 mL hexane (< 2 mL/min) and retained for future PBDE analysis. The second fraction was eluted with 10 mL DCM (< 2mL/min) and concentrated to dryness, reconstituted in 200 µL methanol. The final extract was transferred to inserted glass amber vials and analysed for HBCDs via LC-ESI-MS/MS.

Results and discussion

The concentrations of sum HBCD (α,β and γ HBCD) in human blood serum collected in 2010/11 in Australia ranged from not detected to 37.17 ng/g lipid (Table 1). The mean, median and 95th percentile for sum HBCDs were 4.44, 0.77 and 12.56 ng/g lipid, respectively.

Table 1. Concentration of sum HBCD (α,β and γ HBCD) in ng/g lipid for males and females by mean age (years)

Males		Females	
Age	Conc. (ng/g lipid)	Age	Conc. (ng/g lipid)
2	0.62	2	n.d.
2	0.59	2	n.d.
10	0.73	10	13.13
10	n.d.	10	n.d.
23	0.54	23	1.15
23	n.d.	23	9.33
38	37.17	38	0.81
38	1.58	38	9.04
53	0.66	53	6.11
53	n.d.	53	6.83
70	3.26	70	7.43
70	n.d.	70	7.53

While the results from this study show variable results with little correlation between replicate strata and no age trends, sum HBCD concentrations were higher in females than in males. Specifically, the pools for females aged 5 – 15, 16-30, 31-45 and 45-60 years show high variation at between 5 and 18 times the concentrations in males. The lack of obvious age trend is in contrast to concentrations of the flame retardants – polybrominated diphenyl ethers (PBDEs) where concentrations are highest in children and plateau around 20 years [14]. It should be noted there was one outlier in the males, 31-45 years which may have been due to one or a few donors in the blood serum pool having recent high exposure, therefore increasing the 'average' concentration in the

pool. The variation noted in these samples could also be due to the human half-life of HBCD which is estimated to be 64 days (0.17 years) in comparison to PBDEs which ranges from 1.6 to 6.5 years.

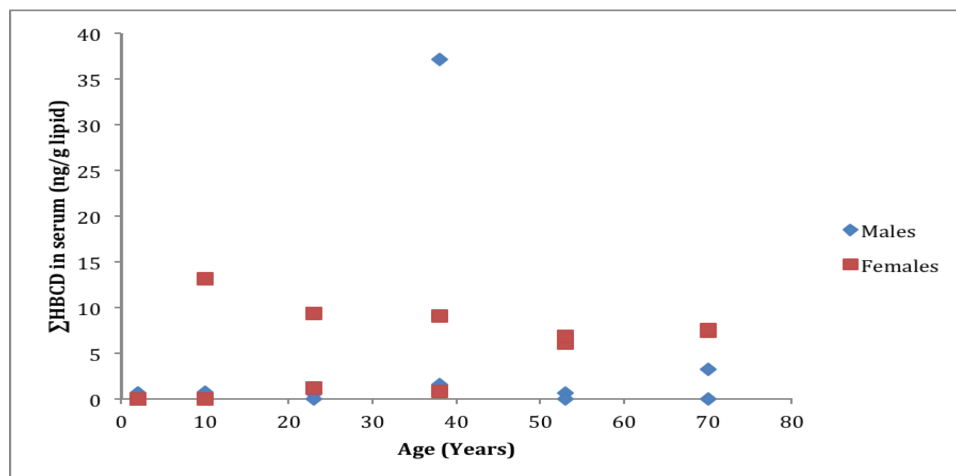

Figure 1. Sum HBCD (ng/g lipid by mean age for males and females)_

The diastereoisomeric pattern showed a predominance of the α- and γ-isomers contributing between 60-100% and 23-40% to the sum HBCD concentration, respectively, with the β-isomer was below the limit of quantification in all but one sample. The diastereoisomeric pattern may be due to individual variability in metabolizing capacity or the frequency of HBCD exposure as well as more extensive metabolism of β- and γ-HBCD diastereoisomers in organisms than α-HBCD. Differing diastereoisomeric patterns have also been observed with α-HBCD the most abundant stereoisomer in serum samples from Sweden [15], Canada [16] and Japan (Kakimoto et al. (2007) while Johnson-Restrepo et al. [17] reported that γ-HBCD was the dominant isomer present in the U.S.

In comparison to HBCD concentrations in breast milk from Australia. The concentrations of total HBCD which ranged from nq to 19 ng/g lipid which is similar to that found here for females were the range was not detected up to 13.13 ng/g lipid. In Canada, HBCD concentrations were lower than in Australia ranging from 0.33 – 8.9 ng.g lipid [18].

For HBCD, the results from this study are in agreement with international data showing variability in both the diastereoisomeric and enantiomeric patterns and are indicative of individual differences in either exposure or metabolism of this chemical. This was the first study of HBCD in human blood serum in Australia. Continued monitoring over time with the use of individual samples of human blood serum for HBCD is warranted to further assess and monitor the body burden of BFRs in the Australian population. An assessment of source or exposure data on HBCD from Australia would contribute valuable information to the investigation of HBCD body burden in Australia.

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