TRENDS OF THE BROMINATED FLAME RETARDANTS, PBDES AND HBCD, IN HUMAN MILKS FROM NORTH AMERICA

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Introduction

In the last decade human exposure to brominated flame retardants (BFR) has been documented worldwide particularly in developed countries[1-4]. Concentrations of polybrominated diphenyl ethers (PBDEs) in human milk are known to be significantly higher in USA and Canada than in other industrialized countries in Europe and Asia- median values of total PBDEs in North America expressed on a milk lipid basis are at least an order of magnitude higher than elsewhere[2,3]. In 2004-5, we sampled human milks from two areas of North America from which we had previously generated PBDE milk data in 2002 and earlier. Our goal was to determine whether the contemporary levels of PBDEs in Hamilton, Ontario, Canada and Austin, Texas, U S A, already some of the highest in the world, had changed in the interim.

Hexabromocyclododecane (HBCD) is a related additive BFR widely used in polystyrene foams for insulation and for textile backings. It is claimed to be the third most commonly manufactured BFR by amount whose use is believed to be increasing due to environmental pressures to reduce PBDEs. HBCD occurs as several diastereoisomers with the commercial product containing more than 98 % of the γ-isomer. Although limited data on human exposure to HBCD exists in Europe, this BFR consists of several isomers and, to date, no information is available on its isomeric content in humans. In this paper we analysed both the current North American milk samples for PBDEs and we also determined HBCD in these and earlier collected samples using an isomer specific method in order to obtain information on the occurrence of HBCD in lactating women.

Materials and Methods

Human milks were obtained in April 2005 from McMaster University Hospital, Hamilton, Ontario and in October 2004 from the Mothers Milk Bank in Austin, Texas. Approval for sample collection from the donor mothers was obtained from the research ethics boards of these two groups as well as that of Health Canada. Analysis of the milks for PBDEs was carried out by spiking with carbon-13 congeners and using solvent extraction, lipid removal, followed by purification and fractionation on Florisil and carbon. Determination was by GC-MS in the high resolution electron impact mode with isotope dilution for quantification[4,5]. Milk values are usually expressed on a milk lipid basis- the latter content being determined gravimetrically after organic solvent extraction.

LC-MS

In the cleanup procedure, HBCD tracks with the PBDEs in the same purified fraction although HBCD is slightly more polar. This purified fraction was also used to measure HBCD and, for the first time, an isomeric determination of HBCD in human specimens was carried out. Each milk sample was spiked initially with 2-10 ng each of the three fully mass labelled carbon-13 isomers, α-, β-, and γ-, as surrogate standards, $^{13}C_{12}H_{18}Br_6$, (Wellington Laboratories, Guelph, Canada). After purification as above for the PBDEs, the BFR fraction containing both HBCD and PBDEs was dissolved in a mixture of 100 uL methanol- water (4:1). LC-MS was carried out using a VG Quattro 2 MS

coupled to a HP 1100 HPLC. These conditions are somewhat modified from an earlier report[6]. Separation was effected on a C18 reversed phase high pressure liquid chromatographic analytical column (Jones Genesis; 5.0 cm long, 2.1 μm id, 3 μm particle size) beginning with a 60:40 water:methanol-acetonitrile (1:1) as eluent followed by a gradient of increasing amounts of the organic phase at a flow rate of abut 0.2 mL per min. These conditions result in baseline separation of the three main isomers. Detection and measurement were effected by MS/MS with electrospray ionization in the NCI mode. The MRM transition of M-H^- (m/z 640.7 and 638.7) to the Br^- 80.8 and 78.8 isotopes, respectively, was monitored along with the two corresponding transitions (652.7 and 650.7) for the three carbon-13 surrogates. Completely deuterated α-, β-, and γ-HBCD, $^{12}C_{12}{}^{2}H_{18}Br_6$, were used as recovery and performance standards and these internal standards were monitored at 661.5 and 657.4 to the Br- transition at 80.8 m/z. Under these conditions, the on column detectability of each isomer is about 20 pg and the method detection limit on a milk lipid basis for 10-20 mL of sample is about 0.1 to 0.3 μg/kg (ppb). For each group of 8-10 unknown samples, a quality control milk sample and a laboratory blank were processed. The latter showed the sporadic occurrence of small amounts of the γ-isomer.

Health Canada participated in 2006 in an interlaboratory study on the measurement of HBCD in fish organized by the Norway Institute of Public Health. Our results for these two biotic samples containing mostly the α-isomer of HBCD were within 15 % of the median values of all 10 reporting laboratories.

Results and Discussion

Polybrominated diphenyl ethers (PBDEs)

Median levels of total PBDEs in 34 milks collected in 2005 from Hamilton, Ontario were lower than levels from milks collected from the same region in 2002 or 2003 (medians 20, 39, and 33 μg/kg (ppb) milk fat with ranges 4-580, 3-960, and 1-960 , respectively) as shown in table 1. Numerically the 2005 milk PBDE values are lower than 2002 or 2003 but this difference is not statistically significant. All of the human milk samples from the years 2002 to 2005 contain significantly and strikingly higher PBDEs than milks collected in 1992 from Ontario ($P<0.001$).

Table 1. Total PBDEs (n=7) in human milks from two locations in North America
values in μg/kg (ppb) on a milk lipid basis

Location	Year	Number Samples	Median	Mean	Range
Ontario	2005	34	20	48	3.7-580
Ontario	2003	13	39	126	3.2-930
Ontario	2002	14	33	139	1.0-960
Ontario	1992	26	3.1	4.8	0.6-26
Texas	2004	25	43	56	8.9-246
Texas	2002	24	44	82	7.7-402

For the 25 milks taken in Texas in 2004, the median value of 43 μg/kg milk fat was almost identical to that from milks taken two years earlier from the same milk bank in the same American city (ranges 9-250 and 8-400 μg/kg,

respectively) with no statistically significant difference in the levels over time. Overall the results suggest that human exposure to PBDEs in North America, while still the highest world wide, has not changed to any degree in the last two to three years.

Hexabromocyclododecane HBCD

As shown in table 2, data for the HBCD content of milks collected in 2002-3 from two different regions indicate the presence of low (0.3-10 μg/kg (ppb)) levels of this BFR, most of which is the α-isomer and not either the β- or γ-isomers, the latter being dominant in the commercial product. The presence of the α-isomer is shown graphically on the LC-MC chromatogram in the figure. It has been established in other biota such as fish, birds and marine mammals that the major HBCD isomer is the α- form and this alteration from the commercial product appears to be true for humans. Whether this difference from the commercial product which contains virtually only the γ-isomer is due to chemical change in the environment or metabolism within the organism is presently unknown.

Table 2. Hexabromocyclododecane in human milks from two locations in North America
values in μg/kg (ppb) on a milk lipid basis

Location	Year	Number samples	Median α-congener	Mean α-isomer	Range
Ontario	2002-3	8	1.6	3.8	0.4-19
Texas	2002	9	0.5	0.5	0.2-0.9

Limited information is available on human internal exposure to HBCD and all of this has been generated by GC-MS meaning that only total HBCD values are available. Thomson et al.[7] reported positive results for HBCD in 49 of 85 human milks collected from Norway between 1993-2001 with a median value of 0.6, a range 0.3 to 20 μg/kg lipid, and a detection limit of 0.3 μg/kg lipid. At the Third International Workshop on BFR in Toronto in June 2004, two reports were given on human exposure to HBCD. Levels of HBCD were reported in human milks, 12 from Mexico and 5 from Sweden[8], and in 90 human blood and cord serum from Dutch mothers and their infants[9] -all dates of collection not given. Typically in these two studies median levels of less than 1 μg/kg lipid were found with the highest values less than 10 μg/kg. These values, both the medians and their range, are quite similar to what we report here on milks from Canada and the United States in 2002-3. It would appear that human exposure to HBCD in many countries is somewhat less than PBDEs as expressed on a lipid basis, perhaps by at least an order of magnitude lower. Moreover the large discrepancy for human exposure to PBDEs between North America and other developed countries does not appear to exist in the case of HBCD. Lastly, this is the first report of the isomeric content of HBCD in humans where the α- and not the β- or γ-isomer predominates.

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Figure. LC-MS chromatogram of HBCD in human milk from Ontario showing: (i) the carbon-12 (upper two frames) consisting mostly of the α-isomer and (ii) the carbon-13 (lower two frames) surrogate α-, β-, and γ-isomers.