Environment International 70 (2014) 1–8

Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

The brominated flame retardants, PBDEs and HBCD, in Canadian human milk samples collected from 1992 to 2005; concentrations and trends

John Jake Ryan *, Dorothea F.K. Rawn

Bureau Chemical Safety, Health Products and Food Branch, Health Canada, Ottawa, Ontario, Canada

ARTICLE INFO

Article history:
Received 28 January 2014
Accepted 29 April 2014
Available online xxxx

Keywords:
Biomonitoring
Human milk
Brominated flame retardants (BFRs)
Polybrominated diphenyl ethers (PBDEs)
Hexabromocyclododecane (HBCD)

ABSTRACT

Human milk samples were collected from individuals residing in various regions across Canada mostly in the years 1992 to 2005. These included five large cities in southern Canada as well as samples from Nunavik in northern Quebec. Comparative samples were also collected from residents of Austin, Texas, USA in 2002 and 2004. More than 300 milk samples were analysed for the brominated flame retardants (BFRs), PBDEs and HBCD, by extraction, purification and quantification using either isotope dilution gas chromatography-mass spectrometry (GC-MS) or liquid chromatography-MS. The Canadian total PBDE values in the years 2002–2005 show median levels of about 20 μg/kg on a lipid basis; a value significantly higher than in the 1980s and 1990s. Milk samples from Inuit donors in the northern region of Nunavik were slightly lower in PBDE concentrations than those from populated regions in the south of Quebec. Milk samples from Ontario contained slightly lower amounts of PBDEs in two time periods than those from Texas. HBCD levels in most milk samples were usually less than 1 ppb milk lipid and dominated by the α-isomer. This large data set of BFRs in Canadian human milk demonstrates an increase in the last few decades in human exposure to BFRs which now appears to have stabilized.

1. Introduction

Flame retardants are effective and valuable industrial chemicals which reduce the risk from fatal fires particularly in developed countries. Among the many classes of chemicals used for this purpose, the brominated flame retardants (BFRs) are particularly prominent. Two of these, the polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecane (HBCD), have come under regulatory and societal scrutiny due to their persistence and ubiquitous occurrence in the environment including people (Covaci et al., 2006; Frederiksen et al., 2009; Gill et al., 2004; Hites, 2004). The detailed examination of BFRs in the last decade is due, in part, to the seminal papers of Swedish scientists (Meironyté et al., 1999; Norén and Meironyté, 2000). Using human milk samples collected in the early 1970s till the late 1990s in the area of Stockholm, they showed that the concentrations of most of the persistent organic pollutants (POPs) such as PCBs, PCDD/PCDFs, DDTs, and chlordanes decreased by at least a factor of two. A notable exception to this downward trend was the PBDEs which increased steadily in the time period and reached levels on a milk lipid basis of 3–4 μg/kg. Since this important finding, there have been many reports worldwide on the PBDE content in human tissue in general and human milk in particular, a food of nutritional, physiological and psychological importance. Most of these studies have originated from developed countries where BFRs are widely used for the control of accidental fires.

PBDEs as flame retardants were manufactured as three types named after the major bromine congener content of each mixture and designated as penta-, octa-, and deca-. All three types are additive BFRs and were applied in large amounts to electronics, building and household materials including polyurethane foam products. New uses of PBDEs are now banned in most countries although there exist large reservoirs of these compounds in the environment. Concentrations of PBDEs in human milk are known to be significantly higher in North America, specifically USA and Canada, than in other industrialized countries such as Europe and Asia (Ryan and Patry, 2000; Ryan et al., 2002; Schecter et al., 2003; She et al., 2007). Median values of total PBDEs in North American human milk taken in the late millennium and expressed on a lipid basis are in the range of 20–50 μg/kg; values which are up to an order of magnitude higher than elsewhere worldwide.

HBCD is a related additive BFR widely used to treat polystyrene foams for insulation and textile backings and is claimed to be the third most commonly manufactured BFR (Covaci et al., 2006; Marvin et al., 2011). Its use is believed to be increasing due to environmental

Abbreviations: BFR, brominated flame retardant; PBDE, polybrominated diphenyl ether; HBCD, hexabrominated cyclododecane; PCB, polychlorinated biphenyl; no, non *ortho*; mo, mono *ortho*; T, tetra; Pn, penta; Hx, hexa; Hp, hepta; O, octa; POPs, persistent organic pollutants; ND, non detected usually followed by the limit of detection (LOD) in parenthesis.

* Corresponding author at: 251 Banting Dr., Tunney's Pasture, Ottawa, ON K1A 0K9, Canada. Tel.: +1 613 957 0978; fax: +1 613 941 4775.

E-mail addresses: jake.ryan@hc-sc.gc.ca (J.J. Ryan), thea.rawn@hc-sc.gc.ca (D.F.K. Rawn).

http://dx.doi.org/10.1016/j.envint.2014.04.020
0160-4120/

pressures to reduce exposure to PBDEs. Unlike PBDEs which are a mixture of up to 209 congeners and isomers, HBCD occurs as several diastereoisomers with the commercial product containing more than 98% of the γ-isomer with minor amounts of the α- and β-isomers and trace amounts of two other isomers, delta (δ) and epsilon (ε). Similar to PBDEs, global production of this BFR has been banned in 2013 (Hogue, 2013). Data on human exposure to HBCD is more limited than that for the PBDEs but most reports show human milk levels around 1–5 μg/kg lipid. Information on its isomeric content in humans is also limited but suggest that the α-isomer predominates.

PBDEs do not exhibit dioxin-like activity, are not acutely toxic, nor are they believed to be carcinogenic. They do have similar physical, chemical and biological properties as PCBs and are often compared to them. Two areas of considerable scrutiny in the toxicology of both these classes of compounds are endocrine disruption as evidenced by thyroid function changes and neurotoxicology as shown by cognitive and motor effects in experimental animals. However, in studies of the effects of PBDEs on humans, reports of hormonal or developmental dysfunction are often inconsistent or any difference noted is small and of uncertain clinical significance (Eggesbø et al., 2011; Eskenazi et al., 2013; Herbstman et al., 2010). The health consequences of exposure to brominated flame retardants have recently been assessed by Kim et al., 2014 who concluded that the evidence to date was only suggestive of harm.

In this paper we give the detailed PBDE and HBCD results for the analyses of approximately 300 samples of human milk from Canada collected mostly in the period from 1992 to 2005. In addition we compare these BFR data with: 1) even earlier milk samples from Canadian residents, 2) a remote northern region and southern region within Canada, and 3) contemporarily collected similar milk results from the USA. Some of this information has appeared in summary and abbreviated form at scientific meetings (Pereg et al., 2003; Ryan and Patry, 2000; Ryan et al., 2002, 2006). The data are also compared between countries and regions; most notably between North America and both Europe and Asia.

2. Materials and methods

2.1. Sampling

For the collection period in 1992, human milks were obtained from regional provincial health units by the then Field Operations Directorate of Health Canada (Newsome et al., 1995). Three to four weeks after birth, healthy donor mothers resident in Canada for at least five years were requested over a period of about one day to express manually four ounces (about 125 mL) of their milk into a laboratory provided clean glass container. From the 497 samples received, 72 of these were selected for determination of PBDEs. This selection was based on the proportionate population of that particular Canadian province and availability of sample due to prior aliquoting for previous analyses.

For the collection of the samples from individuals in the 2002 period, the 98 donors were recruited on an *ad hoc* basis from the four main southern regions of Canada as follows. Twenty samples were obtained in early 2002 from the British Columbia Women's Milk Bank at the Women's Hospital and Health Centre in Vancouver, BC. Twenty samples were also obtained in 2001–2 from hospital clinics in Edmonton, Alberta. The eighteen samples from Ontario obtained in 2002 as well as the 34 samples in 2005 all originated from the hospital clinic at McMaster University, Hamilton. The Quebec human milks were obtained from two community centres, twenty each, one in Montreal in July 2002 and the other in Quebec City in April 2002. The Nunavik (northern Quebec) samples were obtained from Inuit establishments on the east coast of Hudson Bay area of northern Quebec 1989–1990 (n = 20) and in 1996–1999 (n = 20) (Pereg et al., 2003). The Texas USA samples were collected in 2002 and 2004 from the mother's milk bank at Austin, Texas.

For the 2005 milk samples, the milk collection and program were approved by the research ethics boards of both Health Canada and McMaster University. All milk samples were frozen after sampling and shipped in that state to Ottawa and stored at −70 °C, prior to defrosting and chemical analyses.

2.2. Analysis

Individual milks (2–20 mL) were analysed for a wide range of POPs including the PBDE congeners with two to seven bromines and the three common isomers of HBCD. In summary, the method involved the addition of a mixture of ^{13}C isotopically labelled PBDEs containing seven of the most common PBDE congeners and the three major isomers of HBCD. Milks were extracted with a mixture of acetone-hexane and, after solvent evaporation and drying, the lipid content was determined gravimetrically. The extract was then redissolved in hexane and the lipid was degraded and removed by partitioning with strong sulfuric acid. Further purification of the extract was accomplished on adsorbant columns of acidified silica and activated Florisil (magnesium silicate). Virtually all of the PCBs except the non *ortho* congeners eluted in the first non polar hexane fraction on Florisil while the PBDEs and HBCD eluted in the second more polar dichloromethane fraction along with the PCDD/Fs and the non *ortho* PCBs. The BFRs were separated from the latter compounds by a carbon column which adsorbs the planar compounds but not the BFRs and the latter dissolved in 10 μL toluene and 1 μL injected on GC for mass spectrometric determination (Needham et al., 2002; Ryan et al., 1993, 2006; Schecter et al., 2003).

2.3. GC-MS

Separation of the PBDE congeners was accomplished by on-column injection on a capillary gas chromatographic column 15 m long containing a non polar 0.1 μm film thickness DB-5 bonded phase silicone coating. The GC was coupled to a high resolution (8–10 K) mass spectrometry, either a Kratos Concept or Micromass Ultima AutoSpec, operating in the electron impact (EI) mode at 40 eV. Quantification was by the isotope dilution technique using selected ion monitoring (SIM) acquisition of the di- to hepta- bromo ions and a variable eight point standard calibration curve (Ryan and Rawn, 2014). The calibration standard for PBDEs contains more than 30 congeners some of which are not detected in human tissue samples. Detection limits for the PBDEs depend more on the laboratory blank values than instrumentation detectability; typical values for a 5–10 mL milk sample are in the order of five to 20 ng/kg milk lipid.

2.4. LC-MS/MS

In the cleanup procedure, the fraction not absorbed by the carbon column contains both the PBDEs and HBCDs although the HBCDs are a little more polar. After injection of an aliquot (1 μL of 10 μL) of this BFR fraction on GC-MS, the remaining sample extract was evaporated to dryness and redissolved in a mixture of 100 μL methanol-water (4:1). Injection of 20 μL of the 100 μL extract was carried out with LC-MS/MS using a VG Quattro 2 MS 778 coupled to a Hewlett Packard 1100 HPLC. Separation was effected on a C18 reversed phase high pressure liquid chromatographic analytical column (Jones Genesis; 5.0 cm long, 2.1 μm id, three μm particle size) beginning with a three parts water and two parts methanol-acetonitrile (1:1) as eluent followed by a gradient of increasing amounts of the organic phase at a flow rate of about 0.2 mL per min. These conditions result chromatographically in baseline separation of the three main HBCD isomers. Detection and measurement were performed by MS/MS with electrospray ionization in the negative mode. The multiple reaction monitoring (MRM) transition of $M\text{-}H^-$ (m/z 640.7 and 638.7) to the Br^- 80.8 and 78.8 isotopes, respectively, was monitored along