DETECTION OF POLYBROMINATED DIBENZO-P-DIOXINS AND FURANS (PBDD/Fs) IN HUMAN TISSUE FROM SWEDEN

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Introduction

The increased usage of brominated flame retardants (BFRs) has raised the level of environmental concern regarding brominated dibenzo-*p*-dioxins and furans (PBDD/Fs). It is likely that human, as well as wildlife, exposure to brominated dioxins and furans will increase with this increased usage and waste disposal of BFR products[1]. The PBDD/Fs are unintentional byproducts in PBDE-mixtures[2], and from photolytic[3,4], and thermal[5-7] degradation of BFRs. PBDD/Fs exhibit similar properties, as well as bioaccumulation and toxicity as their chlorinated homologues (PCDD/Fs)[1,8]. PBDD/Fs have been detected in ambient air[9-12], at electronic waste dismantling areas[13], in plastics from TV sets[14], flue gases[15,16], fly ashes[17-20], sediments[10,21,21], diet samples[23], shellfish[24], fish[25], human adipose tissue[26] and milk[27,28], and in blood from occupationally exposed persons[29].

In this study, human adipose tissue from nine individuals, representing the general Swedish population and serum from six individuals exposed to high levels of PBDD/Fs and PBDEs during a fire of high level bromine containing material, were analyzed for PBDD/Fs.

Materials and Methods

Human adipose tissue samples were collected during 2007 at Örebro University hospital. Serum samples corresponding to 30-50 ml whole blood were collected in March 2008. Samples were stored at -20°C. PBDD/F standards; 4-MoBDF, 2,7-DiBDF, 2,8-DiBDF, 2,3,8-TriBDF, 1,2,7,8-TeBDF, 1,2,3,7,8-PeBDF, 1,3,4,7,8-PeBDF, 1,2,3,4,7,8-, 1,2,3,7,8,9-HxBDD, 1,2,3,4,7,8-HxBDF, 1,2,3,4,6,7,8-HpBDF, OBDD, and OBDF were purchased from Wellington Laboratories Inc., Guelph, Canada, and ^{13}C-labeled 2,3,7,8-TeBDF, 1,2,3,7,8-PeBDF, 2,3,4,7,8-PeBDF and 1,2,3,4,7,8-HxBDF were from Cambridge Isotope Laboratories Inc., Andover, MA, USA. Organic solvents used were of pesticide grade and purchased from Riedel de Haën (n-hexane, dichloromethane, and toluene).

Adipose tissue and serum samples were ground with anhydrous sodium sulfate. Open column chromatography was applied for approximately five gram adipose tissue and ten gram serum. Sample clean-up was performed using three open columns (multilayer silica, AlOx and active carbon). The multilayer silica column, containing KOH silica, neutral activated silica, 40% H_2SO_4 silica gel, 20% H_2SO_4 silica gel, neutral activated silica gel, and anhydrous Na_2SO_4, was eluted with hexane. This column was followed by an AlOx column eluted with hexane/dichloromethane. Additional clean up and fractionation was performed using active carbon (Carbopack C dispersed on Celite 545), eluted with 10 ml of hexane for non-planar compounds and 80 ml of toluene for eluting the planar fraction containing PCDD/Fs and PBDD/Fs. Addition of a ^{13}C-labeled recovery standards was done prior to instrumental analysis. Throughout the sample preparation the samples were kept shielded from UV light to avoid photo degradation.

HRGC/HRMS analyses was performed on a Micromass Autospec Ultima operating at 10 000 resolution using EI ionization at 35 eV. All measurements were performed in the selective ion recording mode (SIR), monitoring the two most abundant ions of the molecular bromine cluster. Quantification was performed using the internal standard method. Analyses were performed using two different length columns, a 15 m DB-5MS and 25 m BP-1 column (0.10 mm id, 25 µm) for quantification and verification of retention time match with mass-labeled standards. Ramped on-column injection was used to inject 1 µl of the final extract on the GC column. The GC temperature program started at 120°C, held 2 min and then the temperature was increased by 20°C/min to 260°C, thereafter to 300°C by 10°C/min. The temperature was held at 300°C for

13 minutes and then increased with 20°C/min to 320°C where it was held for 3 minutes. Detection levels were calculated at a S/N ratio of 3, corrected for recovery of the internal standard.

Criteria for determination of PBDFs

Mono- to octaPBDFs were analyzed in all samples. Peaks were identified using ion ratio and the retention time match. The congeners were confirmed by retention times matched with internal standards and isotope ratios within 15 %. To further confirm the identity of PBDFs the following signals were monitored; [PBDF-COBr], [PBDE + 1Br] and [PBDE+2Br]. The fragment obtained when the COBr group leaves the PBDF molecules is formed by EI ionization of PBDFs but not for PBDEs. Monitoring the other fragments is to confirm that the observed PBDFs are not resulting from thermal degradation of PBDEs in the injector or the column.

Result and Discussion

Brominated furans were detected in all Swedish human adipose tissue samples analyzed. Di-substituted congener (2,7/2,8-BDF) was detected in three out of nine samples analyzed, levels were 0.19-0.30 pg/g lipid. Tetra-substituted 2,3,7,8-BDF was detected in all of the nine samples analyzed, levels were 0.27-2.24 pg/g lipid. Two penta-substituted PBDFs were also detected, levels were 0.23-0.89 pg/g lipid for 1,2,3,7,8-BDF, and 0.44-0.54 pg/g lipid for 2,3,4,7,8-BDF. In the chromatograms, there were also a few peaks indicating the presence of other PBDD/Fs, although this could not be confirmed due to lack of ^{12}C-labeled standards. Figure 1 shows 2,3,7,8-TeBDF run on a 25 m BP-1 column, 0.10 µm film thickness.

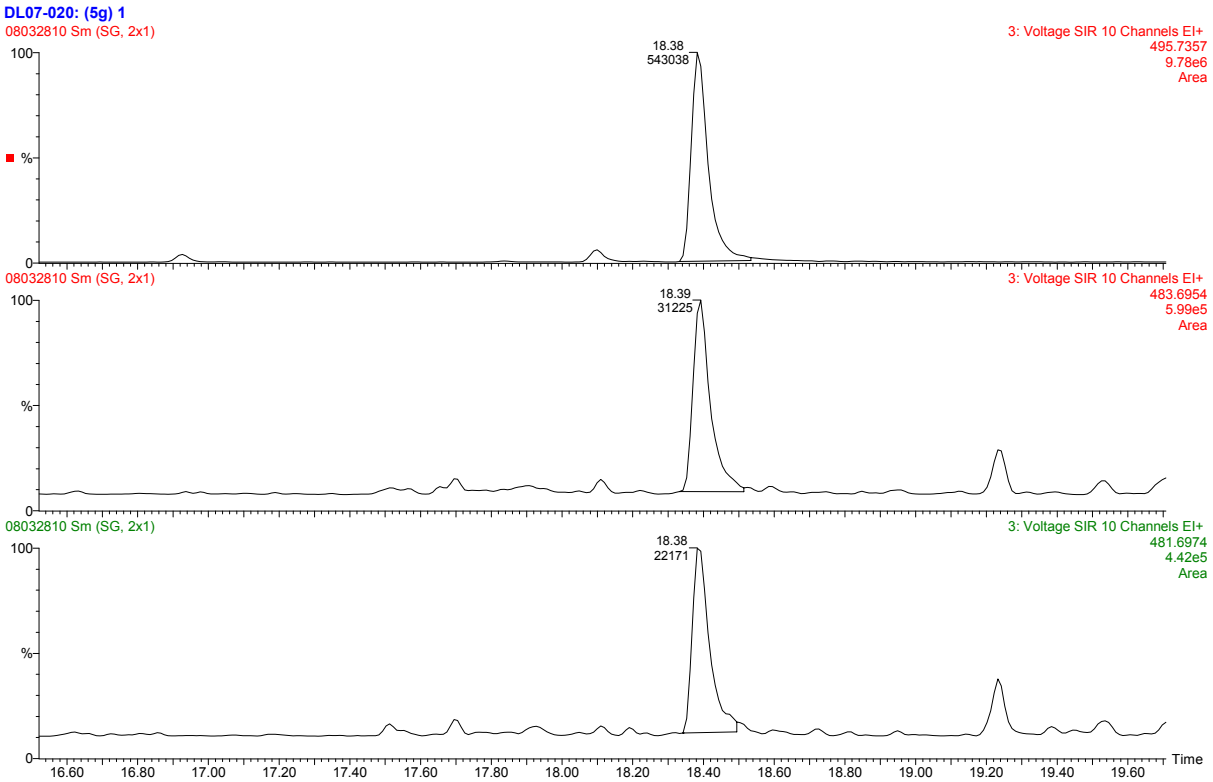

Figure 1. Mass chromatogram of 2,3,7,8-TeBDF in human adipose tissue from Sweden, on a 25 m x 0.1 µm BP-1 column.

The data show the presence of brominated PBDD/Fs in the general Swedish population. The levels of 2,3,7,8-TBDF and 1,2,3,7,8-PBDF are in the same order of magnitude as their chlorinated counterparts. Considering similar TEF-factors as for PCDD/Fs, this might be a source of concern. Currently there are very few reports on PBDD/Fs in human tissue. In 2003, Choi et al[26] published results on PBDD/Fs in Japanese human tissue. The 2,3,7,8-TeBDD congener, 2,3,7,8-TeBDF, and 2,3,4,7,8-PeBDF were found in two sets of samples, with medium concentrations (ranges) for the year 1970 samples 1.7 (<0.8–4.2), 3.3 (1.6–4.3), and 0.31 (0.28–0.60), and for the year 2000 samples 0.51 (0.1–2.0), 2.8 (1.7–4.2), and 0.99

(<0.8-1.9) pg/g lipid respectively, which are similar to the levels found in our samples. In 2006, Kotz[28] also detected the 2,3,7,8-TeBDF and 2,3,4,7,8-PeBDF congeners at concentrations of 0.55 pg/g lipid and 0.33 pg/g lipid respectively, but not the 1,2,3,7,8-PeBDF congener (<0.1 pg/g lipid) in human milk from Sweden collected between year 2000-2003. In addition, 1,2,3,4,7,8-/1,2,3,6,7,8-HxBDF was detected (3.8 pg/g lipid)[28], although no traces of hexaBDFs could be seen in human adipose in this study. The three congeners detected by Choi et al[26] in 2003 are the same as the most dominant congeners found in ambient air samples from an electric waste dismantling area in China[13] as well as from various locations in Shanghai[30] suggesting that the large scale usage of BFRs could possibly be one source to the elevated levels of PBDFs in human adipose tissue from Sweden.

Table 1. Levels (pg/g lipid) of PBDD/Fs in nine Swedish human adipose tissue samples.

age	Female 61	Male 61	Female 53	Male 38	Female 41	Male 54	Female 21	Male 48	Male 65
Furans	pg/g	pg/g	pg/g	pg/g	pg/g	pg/g	pg/g	pg/g	pg/g
4-MoBDF	<0.07	<0.08	<0.08	<0.09	<0.07	<0.08	<0.08	<0.06	<0.09
2,7/2,8-DiBDF	<0.10	0.30	<0.11	<0.12	<0.10	<0.11	0.12	0.14	<0.12
2,3,8-TriBDF	<0.04	<0.08	<0.11	<0.14	<0.14	<0.19	<0.21	<0.19	<0.03
2,3,7,8-TeBDF	2.24	0.65	0.54	0.49	0.69	0.71	0.41	0.27	0.80
1,2,3,7,8-PBDF	0.89	<0.11	<0.11	<0.12	0.29	<0.11	0.23	<0.08	<0.12
2,3,4,7,8-PBDF	0.54	<0.11	<0.11	<0.12	<0.10	<0.11	<0.10	<0.08	0.44
Dioxins									
1-MoBDD	<0.07	<0.08	<0.08	<0.09	<0.07	<0.08	<0.08	<0.06	<0.09
2,7/2,8-DiBDD	<0.07	<0.08	<0.08	<0.09	<0.07	<0.08	<0.08	<0.06	<0.09
2,3,7-TrBDD	<0.02	<0.03	<0.03	<0.03	<0.02	<0.03	<0.03	<0.02	<0.03
2.3.7.8-TeBDD	<0.04	<0.05	<0.05	<0.06	<0.05	<0.05	<0.05	<0.04	<0.06
1.2.3.7.8-PeDD	<0.16	<0.19	<0.19	<0.20	<0.17	<0.19	<0.18	<0.14	<0.21

Because of the small sample size (<0.5 g lipid) no PBDD/Fs were detected in the serum samples from the individuals occupationally exposed to a fire of bromine containing pharmaceuticals. In one of the samples, comprising the largest amount of serum, peaks corresponding to 2,3,7,8-TeBDD and other TeBDDs could be seen, but peaks did not pass our QA/QC criteria for positive identification. However, levels in these samples are not expected to exceed limit of detection (LODs) as reported in Table 1.

Conclusions

Brominated PBDFs (2,7/2,8-DiBDF, 2,3,7,8-TeBDF, 1,2,3,7,8-PBDF, and 2,3,4,7,8-PBDF) were found in human adipose at concentrations of 0.12 – 2.24 pg/g, being the first results on a larger material of the general population in Sweden.

Acknowledgements

The Swedish EPA is acknowledged for their financial support, contract nr 219 0603, dnr 721-1610-06Mm. SGE Australia is acknowledged for making the BP-1 column available.

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