Determination of polybrominated diphenly ethers in human adipose tissue by selective reaction monitoring from Qatari's population.

Murad Helaleh[1*], Nada Al-Tamimi[1], Aishah Latiff[1], Mohamed El-Rayes[2], Thomas M.H[3]

[1]Anti Doping Lab, Toxicology and Multi-purpose Lab., P.O Box: 27775, Doha, Qatar

[2]Anti Doping Lab, life Science, P.O Box: 27775, Doha, Qatar

[3]Anti Doping Lab, Risk Quality, P.O Box: 27775 , Doha, Qatar

Introduction

Polybrominated diphenyl ethers (PBDEs) are a class of brominated flame retardants (BFR), accounting for 33% of the worldwide BFR production in 2001. They are listed as priority substances within the European Union Water Framework Directive, and their occurrence in the environment has been a cause of growing concern. PBDEs have been widely used as flame retardants and was used in polyurethane, foams, computers, paint and plastic to reduce fire risk. PBDEs are tend to bio-accumulate through the food chain. Several studies have reported the occurrence of these chemicals in environmental and human body fat. Detection levels of PBDEs in human is important in order to estimate exposure to these chemicals. Monitoring of PBDEs in humans has been started only recently and no date available from Qatar population. However, PBDEs levels in humans are on the order of nano-gram per gram of lipid weight. Most of the work has been carried out by sensitive systems, such as electron impact selective reaction monitoring mass spectrometry (EI_SRM), were mass of the molecular ion and product ion for each level of brominated is recorded. The advantage of electron ionization mode is to reduce the miss-interpretations of interfering substances and allows the use of 13C-labeled standards (as internal standards) which resulted in obtaining more quantification accurate results. In this context, an analytical method was developed for the simultaneous measurement of tri- to decaBDE from adipose human tissue. Therefore, our primary objectives were: (1) to analyze human adipose tissue specimens from Qatar population, and (2) to explore levels of PBDEs in order to understand the pattern of exposure.

Materials and methods

Pure standards (1.2 ml 1000 ng/ml, 2000 ng/ml and 5000 ng/ml) of each certified standard solutions of PBDE, catalog number: EO-5405 (3, 7, 15, 17, 28, 47, 49, 66, 71, 77, 85, 99, 100, 119, 126, 138, 153, 154, 155, 166, 181, 183, 190, 203, 205, 206, 207, 209) congeners were purchased from Cambridge Isotope Laboratories (Andover, MA, USA). Pure internal standards (1.2 ml 1.0 ug/ml, 2.5 ul/ml, 5.0 ug/ml and 25 ug/ml) of each certified internal standard solutions of PBDE, catalog number: EO-5426 ($^{13}C_{12}$-15,

$^{13}C_{12}$-28, $^{13}C_{12}$-47, $^{13}C_{12}$-99, $^{13}C_{12}$-153, $^{13}C_{12}$-154, $^{13}C_{12}$-183, $^{13}C_{12}$-197, $^{13}C_{12}$-206, $^{13}C_{12}$-209 and $^{13}C_{12}$-209) were purchased from Cambridge Isotope Laboratories (Andover, MA, USA).

Before sample extraction, the internal standards (including ^{13}C-PBDEs) were added into the adipose tissue. Adipose tissue sample was homogenized with anhydrous sodium sulfate/ diatomaceous earth. Aliquot of the extract was evaporated and the residues were weighted for lipid determination. Place the cells filled with the adipose tissue sample and acidic silica (40%) onto the ASE300. Label the appropriate numbers of collection vials and place these into the vial carousel. Set up the extraction method and begin the extraction. Using the following extraction conditions: Solvent: hexane: DCM (9:1); temperature= 80 ^{o}C; pressure= 1500 psi; static time= 5 min; static cycles= 2; flush= 60% and purge= 240 s. The quantification of PBDEs was performed using thermo-TSQ, in an electron ionization (EI) mode with MS-MS. The separation procedure and the monitored ion fragments of all BDE congeners has been achieved by fragment the parent ions and quantified using two parent ions and two daughter ion. For BDE-209 are performed using 15 m column, with a DB-5 column (15 m × 0.25 mm internal diameter and 0.25 μm film thickness). The GC oven temperature was programmed as follows: the initial temperature of 120°C was maintained for 2 min, which was then increased to 250°C at a rate of 25°C min^{-1}, followed by a 15.0 °C min^{-1} increase to 260°C and finally at 25.0 °C min^{-1} increase to 300°C for 15 min. The GC was equipped with a programmed temperature vaporization (PTV) injector run in the split-less mode, which was set at 80^{o}C with a split-flow of 100 ml/min and a split-less time of 1.5 min. The flow rate was 1.2 ml/min with helium as carrier gas.

Results and discussion

The method allows the determination of PBDEs congeners from mono-PBDEs to deca-PBDEs. The five major PBDE congeners (BDE 28, 47, 99, 100, and 153) at concentrations below 1 ng/g lipid weight was achieved.

Among the 28 PBDEs profile, seventeen PBDE congeners were identified (BDEs 28, 66, 71, 77, 100, 119, 85, 155, 154, 153, 138, 166, 183, 181, 191, 205 and 207) in human adipose tissue (subcutaneous and omental tissue). BDE-66 was found to be the dominated congener, followed by BDEs 181, 191, 183, and 138. BDEs 153, 154, 166, 205 and 207 were also detected in most human adipose tissue samples. Total PBDEs concentration (as a sum of all counted congeners) were ranged from 0.18 to 22.94 ng/g lipid weight. Tri- to hepta-BDEs in adipose tissue, the major BDEs congeners monitored (BDEs 28, 66, 71, 77, 100, 119, 85, 155, 154, 153, 138 and 183) were identified in most of the analyzed samples. The total concentration calculated for these congeners ranged from 0.18 to 11.90 ng/g lw. However, for the octa- to deca-PBDEs in adipose tissue, BDE-205 and BDE-207 could be quantified in the analyzed samples, at concentration levels 0.66 ng/g lw and 0.43 ng/g lw. The distribution of the median and the range of the PBDEs among the selected human adipose tissue is presented in Fig. 1.

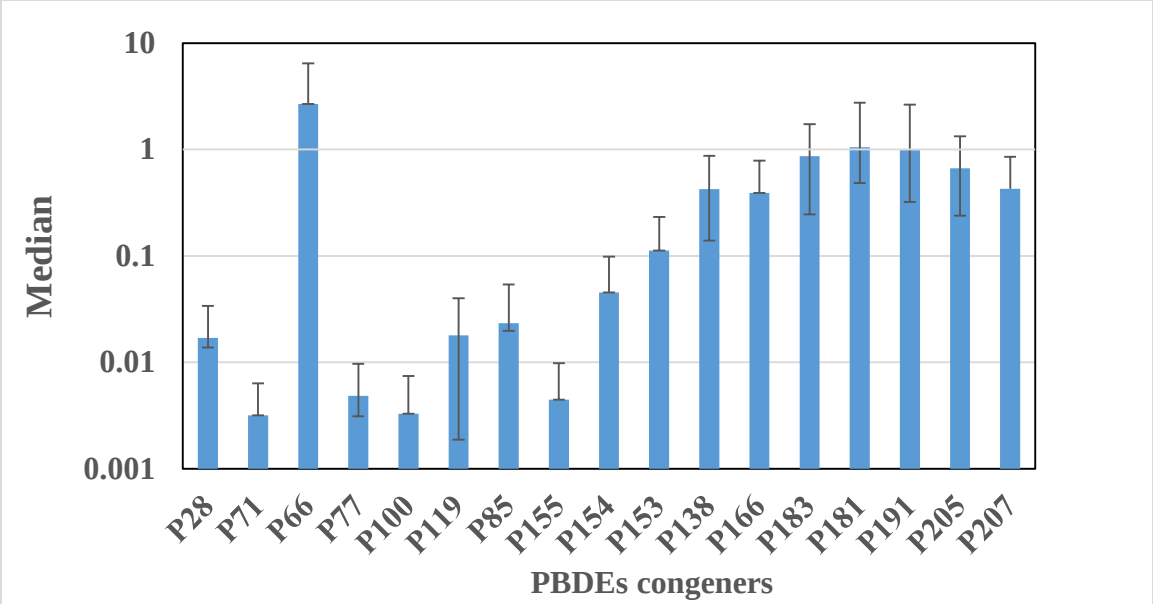

Fig. 1 Distribution of the median and the range of PBDEs among the selected adipose tissue

A chromatogram for the separation of the subcutaneous adipose tissue sample are shown in Fig. 2.

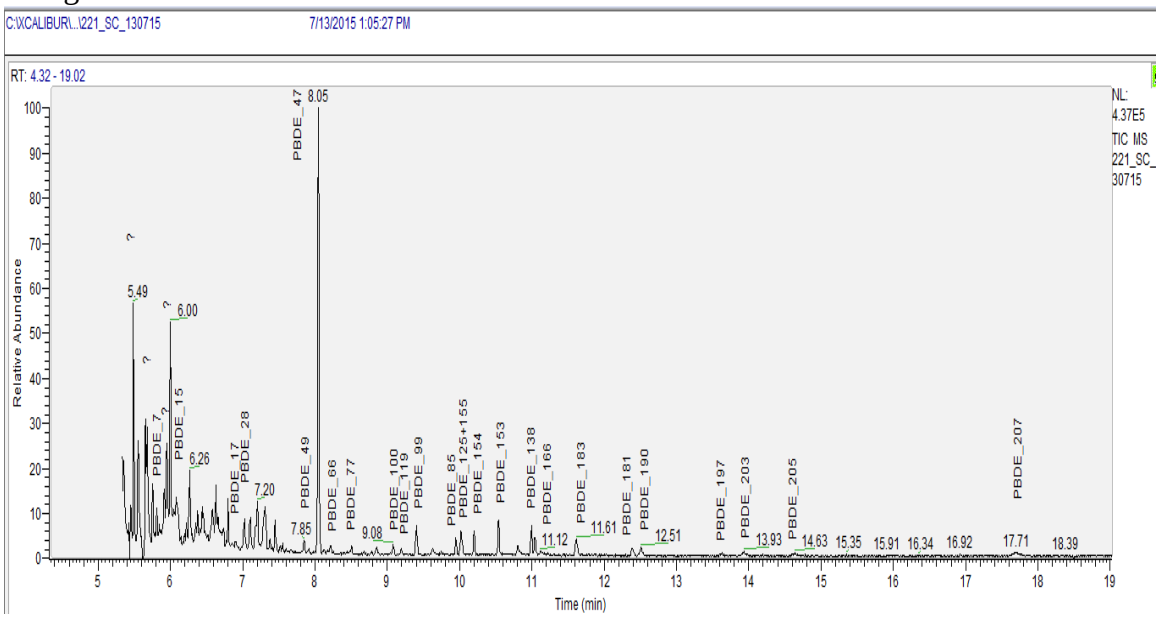

Fig. 2 Chromatogram of subcutaneous adipose tissue sample (221_SC) for the separation of PBDEs using GC-MS- SRM.

The results clearly demonstrate the presence of high molecular weight PBDEs in human adipose tissue. This suggest to conduct a further investigation to carry on related to these substances in order to understand the extend of occurrence in adipose tissue and how they represent a toxicological hazard for human health. The results clearly show the ubiquitous occurrence of PBDEs in the tested samples from Qatar,

This is the first study to report levels of PBDEs in human adipose tissue from Qatar. The results should treated with caution, as they are based on a small sample size. Levels of PBDEs in more than 20 Qatari human adipose tissue samples were analyzed and the results were compared with the values from Europe and rest of the words.

Acknowledgements
The authors are highly indebted to the IREC committee at the Anti Doping Laboratories for financing this project.

References:

1- Kalantzi OI, Brown FR, Caleffi M, Goth-Goldstein R, Petrea M. 2009. Environ Int 35: 113-117.
2- Antignas JP, Cariou R., Zalko D, Berrebi A, Cravedi J-P, Maume D, Marchand P., Monteau F, Riu A, Andre F, Bizec BL. 2009. Environ Poll 157: 164-173.
3- Medina CM, Pitarch E, Portoles T, Lopez FJ, Hernandez F. 2009. J Sep Sci 32: 2090-2102.
4- Cariou R., Antignac J-P, Marchand P, Berrebi A, Zalko D, Andre F, Bizec BL. 2005. J. Chromatog 1100: 144-152.
5- Covaci A, Voorspoels S, Roosens L, Jacobs W, Blust R, Neel H. 2008. Chemosphere 73: 170-175.
6- Pulkrabova J, Hradkova P, Hajslova J, Poustka J, Napravnikova M, Polacek V. 2009. Environ Int 35: 63-68.