Polybrominated diphenyl ethers in human liver and adipose tissues. A pilot study.

Daiva Meironyté Guvenius and Koidu Norén

Department of Medical Biochemistry and Biophysics, Karolinska Institutet,
S-171 77 Stockholm, Sweden

Introduction

Polybrominated diphenyl ethers (PBDEs) are manufactured for industrial use as flame retardants. They are mainly used in polymers designed for, e.g., various electric components, TV-sets and computers, but also in textiles. Theoretically, there are 209 possible PBDE congeners. The manufactured products for use as flame retardants are penta-, octa- and decabromodiphenyl ethers (1). The use of PBDEs has led to an uncontrolled distribution of the compounds into the environment and several PBDE congeners have recently been identified in human blood (2,3), milk (4,5) and adipose tissue (6). In samples from the general population, 2,2',4,4'-tetrabromodiphenyl ether (PBDE-47) was found at highest concentrations. A continuous increase in the levels of PBDEs was found in Swedish human milk from 1972 to 1997 (5), indicating an ongoing pollution. In the present investigation paired samples of liver and adipose tissue were analysed for PBDEs containing 4-6 bromine atom in the molecule.

Material and Methods

Samples

Liver and adipose tissue were collected at autopsy from Swedish subjects, 2 men who had suffered sudden death. The age of the subjects were 78 (A) and 66 (B) years.
The samples were frozen immediately after collection and stored at -20°C.

Chemicals

The standards 2,2',4-triBDE (BDE-17), 2,4,4'-triBDE (BDE-28), 2,2',4,4'-tetraBDE (BDE-47), 2,3',4,4'-tetraBDE (BDE-66), 2,2',4,4',6-pentaBDE (BDE-100) and 2,2',4,4',5,6'-hexaBDE (BDE-154) were kind gift from Prof. Åke Bergman. 2,2',3,4,4'-pentaBDE (BDE-85), 2,2',4,4',5-pentaBDE (BDE-99), 2,2',4,4',5,5'-hexaBDE (BDE-153) and C^{13}-3,3',4,4'-tetraBDE (C^{13}-BDE-77) were purchased from CIL (Andover, MA, USA).

Instrumentation

GC/MS was performed on a VG 70-250 mass spectrometer equipped with an HP 5890A gas chromatograph and a VG-250 data system. A fused silica SE-54 capillary column was used for separation. The column was 25 m x 0.32 mm ID and had a film thickness of 0.25 μm (Quadrex, New Haven, CT, USA). The oven temperature was 190°C for 0.1 min, programmed to 230°C at 4°C/min, hold for 0.2 min, programmed to 235°C at 1°C/min, hold for 0.2 min, programmed to 273°C at 3.5°C/min, and hold for 8 min. The injections were made using an all-glass falling-needle injector. EI was performed in an "EI-only" ion source at the electron energy of 31 eV and

the trap current of 500 μA. The acceleration voltage was 6 kV and the resolution at m/z 293 was 5000 - 7000. The MS was operated in a selected ion monitoring mode (SIM). For each compound, two ions of the molecular ion or fragment ion cluster were monitored.

Analytical procedure

A method previously developed for analysis of organochlorine compounds in human tissues (7) was adapted for analysis of PBDEs. Liver (3g) and adipose tissue (1g) samples were cut into small pieces and weight into a centrifuge tube with a PTFE-lined screw cap. Internal standard C^{13}-BDE-77 (100 μl of 5 pg/μl) was added and samples were homogenised twice with hexane/2-propanol (20 ml, 3/2 v/v), and twice with hexane (10 ml). The combined organic phases were evaporated under reduced pressure. The residue was dissolved in hexane (samples containing more than 0.3 g lipids were diluted with hexane and an aliquot of the sample was taken to further analysis) and extracted by liquid-gel partitioning using Lipidex 5000 and continuous addition of water (8). The gel was transferred to a column and eluted with methanol/water 30/70 (v/v) and 50/50 (v/v). The organohalogen compounds and part of the lipids were eluted with 90 ml of acetonitrile and the remaining lipids with methanol/trichloromethane/ methanol 1/1/1 (v/v).
The acetonitrile fraction was applied on an aluminium oxide column and the organohalogen compounds were eluted with hexane. Further purification and separations were performed by chromatography using silica gel and Bio-Beads S-X3 (5).

Results and Discussion

Samples of liver and adipose tissue from two subjects were analysed for PBDEs. The pattern of PBDEs in adipose tissue and liver was similar in both samples (Figure 1, 2).

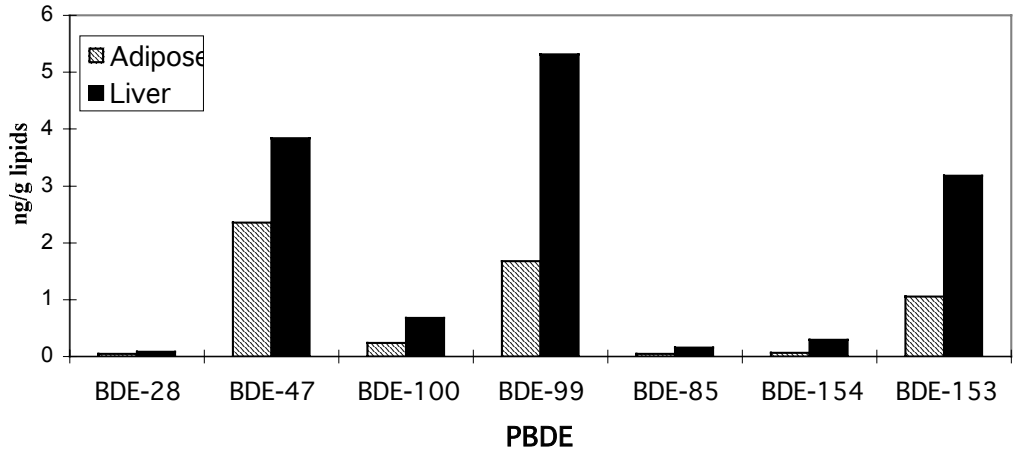

Figure 1. Concentrations of PBDE congeners in liver and adipose tissue from subject A.

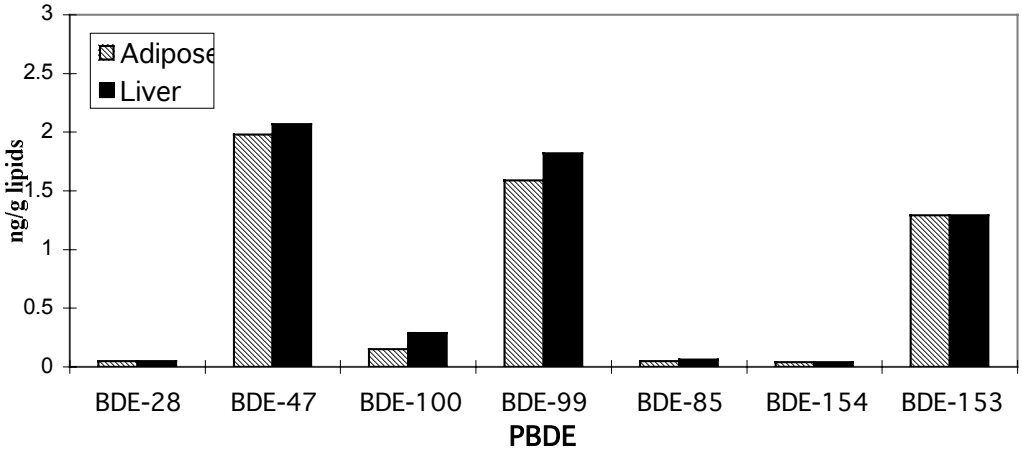

Figure 2. Concentrations of PBDE congeners in liver and adipose tissue from subject B.

BDE-47, BDE-99 and BDE-153 were the predominant PBDE congeners in the two subjects. In subject A the concentrations of PBDEs were higher in liver than in adipose tissue, while in subject B the levels were similar. The concentration of BDE-47 in adipose tissue was 2.4 and 2 ng/g lipids in subjects A and B, respectively. These levels are similar to the levels in human milk from 1996/97 (5), and of the same order of magnitude as previously reported concentrations in adipose tissue from no-cancer individuals (6).
The profile of PBDEs in liver and adipose tissue differed from that in human milk and blood (5,2). The predominant congener in milk was BDE-47 and constituted 60%-70% of the total PBDE amount in samples collected during 1976-1997 (5). The sum of PBDEs in liver samples was 14 and 6 ng/g lipids in subject A and B, respectively. In adipose tissue the sum of PBDEs was 5 ng/g lipids in both cases and similar to the levels in human milk from 1997.
Further analyses are going to be performed to complete the investigation.

Acknowledgements

We are sincerely grateful to Prof. Åke Bergman, dept. Environmental Chemistry, Stockholm University, Sweden for kind gifts of standards. Mistra Foundation for Strategic Environmental Research, Karolinska Institute and Lithuanian Ministry of Education and Science are acknowledged for financial support.

References

1. Environ Health Criteria 162, Brominated Diphenyl Ethers. World Health Organisation, Geneva, **1994.**
2. Klasson Wehler E., Hovander L. and Bergman Å. *16th Symposium on Chlorinated Dioxins and Related Compounds. Organohalogen Compounds*. **1997**, 33, 420.
3. Sjödin A., Hagmar L., Klasson Wehler E., Kronholm-Diab K., Jakobsson E. and Bergman Å. *Environ Health Perspect*. **1999**, accepted.

4. Darnerud P., Atuma S., Aune M., Cnattingius S., Wernroth M. and Wicklung-Glynn A. *18th Symposium on Halogenated Environmental Organic Pollutants. Organohalogen compounds*. **1998**, 35, 411.
5. Meironyté D., Bergman Å. and Norén K. *J Toxicol Environ Health* .**1999**, submitted.
6. Lindström G., Hardell L., van Bavel B., Wingfors H., Sundelin E., Liljegren G. and Lindholm P. *18th Symposium on Halogenated Environmental Organic Pollutants Organohagalogen Compounds*. **1998**, 35, 431.
7. Weistrand C. and Norén K. *Environ Health Perspect*. **1997**, 105, 644.
8. Weistrand C. and Norén K. *J Chromatogr*. **1993**, 630, 179.