CONCENTRATION OF POLYBROMINATED DIPHENYL ETHERS (PBDES) IN THE HUMAN BILE IN RELATION TO THOSE IN THE LIVER AND BLOOD

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Introduction

Polybrominated diphenyl ethers (PBDEs) are used for many plastics products, such as car, textile, television, personal computer, etc. The amount of production has reached 40,000t in 1992. Although the environmental pollution of brominated flame retardants have been a problem in recent years, research about human exposure is seldom performed.

The toxicity of PBDEs is reported to be an antagonist of thyroid-hormone (T4) [1) 2)], and inhibition of aryl hydrocarbon (Ah) receptor, since PBDEs are compounds similar to PCBs and work as antagonist like PCBs [3)].

The purpose of the present study was to establish the method of quantitative analysis for PBDEs in the biological samples, and to investigate human exposure of PBDEs among Japanese.

We have studied PBDEs levels in the bile, blood and liver from 10 autopsy cases, and clarified that seventeen of PBDE congeners, six of TriBDE congeners, four of TetraBDE congeners, four of PentaBDE congeners and three of HexaBDE congeners are present in the bile, and the total level in the bile was generally the same as in the blood and the liver, in terms of per g lipid.

Materials and Methods

Chemicals

Authentic PBDEs and $^{13}C_{12}$ –PBDEs were purchased from Cambridge Isotope Laboratories, Inc. (USA). All solvents and reagents used were of dioxin-analysis grade.

Samples

The bile from gall bladder, cardiac blood and liver tissue were obtained from 10 cadavers at autopsy with the permission of the bereaved families, and were stored at –80 ºC until analysis.

Preparation of samples for PBDEs analysis

1. Blood

About thirty g of cardiac blood was transferred to a 200 ml tube, and 40pg $^{13}C_{12}$-PBDEs were added as described above, and then a lipid fraction containing PBDEs were obtained by extracting with saturated ammonium sulfate (12 ml), ethanol (6 ml) and n-hexane (18 ml), and repeated extraction by n-hexane three times.

The extracts were washed with ultra-pure water and the organic layer was dried over sodium sulfate and evaporated to dryness. The amount of lipid remaining was determined by weight.

2. Liver

About two g of liver tissue was homogenized in the presence of the five fold of sodium sulfate and transferred to a 100 ml tube. Then, $^{13}C_{12}$-isomers were added. A lipid fraction was obtained by extracting with acetone/*n*-hexane (2:1) method [4].

3. Bile

About thirty g of bile was transferred to a 200 ml tube. Then, $^{13}C_{12}$-isomers were added. A lipid fraction was obtained by extracting with acetone/*n*-hexane (2:1) method [5].

Clean-up

Multi-layer column chromatography

Extracted lipid was dissolved in 3ml of n-hexane. The solution was cleaned up on a multi-layer column countering Na_2SO_4 (1.5g), silica (0.9g), $AgNO_3$-silica (3g), silica (0.9g), 22 %(W/W) H_2SO_4-silica (6g), 44 %(W/W) H_2SO_4-silica (4.5g), silica (0.9g), 2 %(W/W) KOH-silica (3g), silica (0.9g) and Na_2SO_4 (1.5g). Before loading of the sample, the column was washed with 100 ml of n-hexane. After the application of the sample, dioxins eluted in the first fraction by 150 ml of n-hexane. Second eluent was 10 % methylene chloride / n-hexane (200 ml) [6]. The flow-rate was 2.5 ml/min. After about 20 µl of n-decane were added, the eluate was concentrated at 40° on a rotary evaporator to a volume of about 2 ml.

Preparation of the sample for HRGC/HRMS.

PBDEs fraction was evaporated on a rotary evaporator to *ca.* 0.5 ml and transferred to a GC autosampler vial tube. The remaining solvent was evaporated under a stream of nitrogen. The walls of the flask were flushed throughly with small volumes of n-hexane, typically decreasing from 10 to 20µl. This solution was added of 20 pg each of the syringe spike ($^{13}C_{12}$–3,3',4,4'-TetraBDE). A 1.5µl volume of sample was injected and analyzed for PBDEs.

Results and Discussion

Levels of PBDEs in the bile, blood and liver

PBDEs amount in the bile, blood and liver are summarized in Table 1. The total pg values per g-lipid were generally the same among the bile, blood and liver at 2952.6±2915.8, 4086.8±4428.1 and 4001.0±3191.0, respectively (Table 1). Among 27 congeners, the contribution of 2,2',4,4'-TetraBDE (#47) and 2,2',4,4',5,5'-HexaBDE (#153) were the most, accounting for more than 70 % of the total level in all these three organs.

Relationship among bile, blood and liver of total PBDEs level

Correlation between total blood level and total bile level is demonstrated in Fig.1. The correlation coefficient r was 0.64, and the regression equation was y=0.424x+1218.2,where y indicates total pg per g lipid of bile, and x is total pg per g lipid of the blood. We also examined the relationship between total blood level and total liver level. As demonstrated in Fig.1, this also showed good correlation, with a correlation coefficient of 0.60, and the relationship can be expressed as y=0.432x+2235.4, where y is total level in term of per g lipid of liver and x is total level pg per g lipid of blood.

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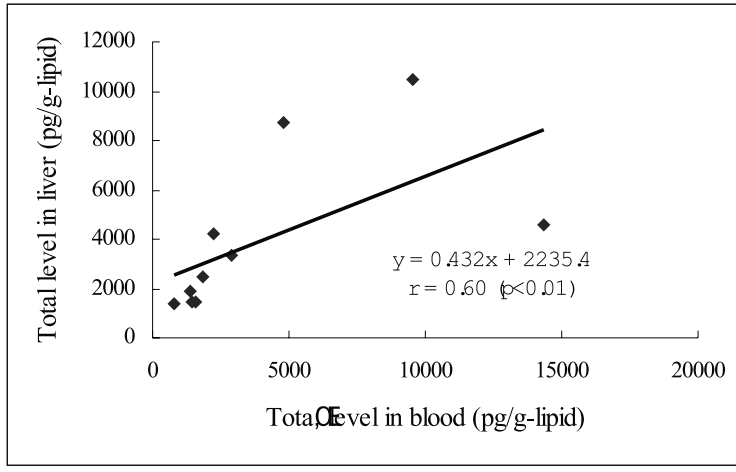
Table 1. PBDE levels in bile, blood and liver

	Mean±SD (pg/g-lipid) (N=10)		
	Bile	Blood	Liver
2,4,6-TriBDE (#30)	N.D.	N.D.	N.D.
2,4',6-TriBDE (#32)	N.D.	N.D.	N.D.
2,3',4-TriBDE (#25)	17.3±29.2	29.1±53.4	32.4±30.1
2,2',4-TriBDE (#17)	5.1±5.3	22.7±28.5	20.7±12.2
2,4,4'-TriBDE+2',3,4-TriBDE (#28+#33)	166.4±170.3	219.0±333.7	240.3±204.9
3,3',4-TriBDE (#35)	1.2±1.4	3.0±3.7	5.7±4.6
3,4,4'-TriBDE (#37)	16.4±19.9	21.7±26.1	24.9±26.7
2,4,4',6-TetraBDE (#75)	N.D.	N.D.	N.D.
2,3',4',6-TetraBDE (#71)	127.5±236.8	130.4±245.6	121.9±118.8
2,2',4,5'-TetraBDE (#49)	N.D.	N.D.	N.D.
2,2',4,4'-TetraBDE (#47)	703.4±827.8	1630.6±2562.7	1382.1±2215.1
2,3',4,4'-TetraBDE (#66)	21.8±26.1	44.4±61.0	32.5±37.8
3,3',4,4'-TetraBDE (#77)	1.7±2.5	5.8±9.3	0.5±1.1
2,2',4,4',6-PentaBDE (#100)	199.6±195.6	285.9±400.1	221.7±126.8
2,3',4,4',6-PentaBDE (#119)	N.D.	N.D.	N.D.
2,2',4,4',5-PentaBDE (#99)	141.0±151.6	256.2±326.8	180.3±163.5
2,3,4,5,6-PentaBDE (#116)	N.D.	N.D.	N.D.
2,2',3,4,4'-PentaBDE (#85)	5.2±16.6	2.6±8.3	N.D.
3,3',4,4',5-PentaBDE (#126)	5.1±16.1	3.9±12.3	N.D.
2,3,3',4,4'-PentaBDE (#105)	N.D.	N.D.	N.D.
2,2',4,4',6,6'-HexaBDE (#155)	45.2±60.6	52.2±104.5	44.6±42.4
2,2',4,4',5,6'-HexaBDE (#154)	70.8±107.2	93.9±223.1	138.0±202.7
2,2',4,4',5,5'-HexaBDE (#153)	1424.9±2151.7	1254.6±1138.7	1554.8±2034.0
2,2',3,4,4',6'-HexaBDE (#140)	N.D.	N.D.	N.D.
2,2',3,4,4',5'-HexaBDE (#138)	N.D.	N.D.	N.D.
2,3,4,4',5,6-HexaBDE (#166)	N.D.	N.D.	N.D.
Total	2952.6±2915.8	4086.8±4428.1	4001.0±3191.0

(N=10)



(N=10)

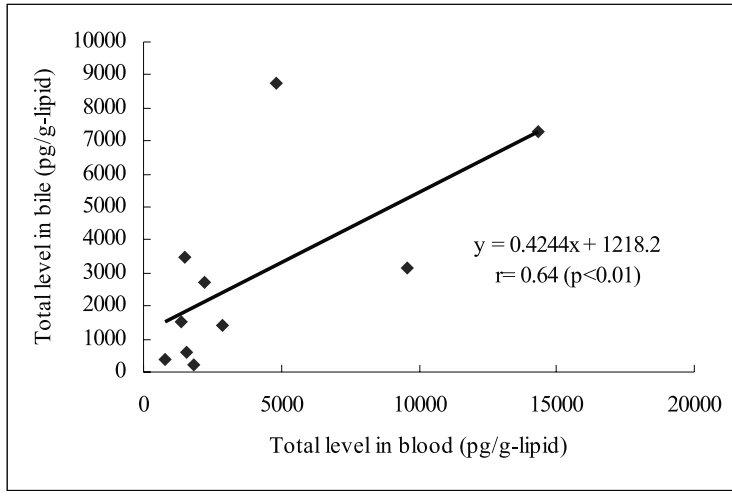


Figure1. Total PBDE levels : Relationship among blood, bile and liver