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TETRABROMOBISPHENOL A AND PHENOLIC XENO-ESTROGENS LEVELS IN INDOOR AIR

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Introduction

Tetrabromobisphenol A (TBBPA) is main brominated flame retardant (BFR). The BFR has drastically increased from 1986 to 1990's and saturated recently in Japan[1]. Recently, it is reported that TBBPA and bisphenol A (BPA) increased the level of the estrogen-regulated proteins, progesterone receptor and pS2, whereas 4,4'-dihydroxybiphenyl showed no such effect[2]. In addition, the human exposure to TBBPA was investigated by the analytical methods for determination of TBBPA in human blood[3].

The report entitled "Global Assessment of the Sate-of-the-Science of Endocrine Disruptors", is the result of a global comprehensive review of the publicly available scientific literature on endocrine disruptors organized by the International Programme on Chemical Safety[4]. In addition, SPEED '98/JEA "Strategic Programs on Environmental Endocrine Disrupters '98" from the Environment Agency, and Health Sciences Researches from the Ministry of Health, Labour and Welfare, have been reported and examined for risk assessment of endocrine disruptors in Japan[5]. Many studies of endocrine disruptors have been investigated at the present time in various countries.

Recently a part of chemicals has attracted a great deal of public attention in Japan which are xenoestrogens as TBBPA, BPA, 4-tert-octylphenol (OP), 4-nonylphenol (NP) and pentachlorophenol (PCP). In the reason, these chemicals have been stayed behind in our life and environment and have a very weak effect for the estrogen response and *in vivo* assay. Take our investigations and studies of the residual existence in our life, trace and high levels of these chemicals were detected. Our studies for monitoring of endocrine disruptors in our life were achieved by the Health Sciences Researches project (1999-2001: a head researcher, PhD. Nakazawa, H.) from the Ministry of Health, Labour and Welfare of Japan. In this a part of researches, the human exposures to TBBPA, BPA, OP, NP and PCP were investigated by the analytical methods for determination of these chemicals in human biological samples. These results shown that very trace levels of some chemicals or metabolites were detected in some human blood or urine sample. Thus, we must make sure the source of these detected chemicals in human biological samples.

Recently many sources of human exposure to these compounds have been existed. High risk factors are some routes by every foods, drink water and indoor air. Therefore, the present study indicates to investigate and evidence of environmental exposure and risk assessment of these compounds as TBBPA in indoor air of Tokyo, Japan. In addition, this approach employed liquid chromatography with an electrospray mass spectrometric detector (LC-MS) and the sampling correction using the stable isotopically labeled internal standards for the sensitive, selective and accurate determination of TBBPA and xeno-estrogens in air sample. This proposed method was

successfully used to determinate TBBPA and xeno-estrogens in indoor air samples from apartment and house in Tokyo, Japan.

Methods and Materials

Reported methods for the measure and sample preparation of phenolic xeno-estorogens in human biological and water samples were modified[6-8]. Liquid chromatography with electrospray mass spectrometry (LC-MS) was performed using an Agilent 1100 MSD-SL system (Agilent Technologies, Palo Alto, USA). The injection volume was 5.0 µl in the needle washing mode. The column used was Mightysil RP-18 GP (150 x 2.0 mm, particle size 5 µm) with a Mightysil RP-18 GP pre-column (20 x 2.0 mm, particle size 5 µm). The column oven was maintained at 40 ^{o}C for the separation of the compounds. The separation was carried out using a mobile phase of 0.01 % acetic acid in water in pure acetonitrile (70/30-[12 min]-30/70, v/v) at a flow rate of 0.2 ml min $^{-1}$. The working conditions for the electrospray MS were as follows: the drying nitrogen gas was set at a temperature of 350 ^{o}C and was introduced into the capillary region at a flow rate of 12 l min^{-1}; the capillary was held at a potential of 3500 V relative to the counter electrode in the negative ion mode. The fragmentor voltage was 140 V during the chromatographic run. When working in the selected ion monitoring (SIM) mode were assigned as the $[M-H]^-$ of this target compounds respectively.

Quantitative analysis was performed using SIM in order to maximize sensitivity. Concentrations were calculated relative to stable isotopically labeled internal standards that were added to the samples prior to sampling and extraction to give a final extract concentration of 2.0 ng ml^{-1} in air extraction samples. Eight-point calibrations were performed daily for all analyzers with internal standards.

The filter disks (glass fiber filter and Empore disk SDB-XD) were conditioned with 50 ml of methanol followed by 30 ml of 5% Ascorbic acid in water and internal standards solutions. Then conditioning, the filter disks were dried. Air sample was passed through the disks at low rate of 7 l/min for 24 hr. Then, 50 ml of water for washing and 30 ml of methanol at a low flow - rate was used to elute the retained compounds. The methanol extracted was evaporated to 0.5 ml with a rotary evaporator. Then, it was adjusted in 1.0 ml of methanol. The obtained samples were measured by LC-MS.

Results and Discussion

LC-MS analytical conditions are reversed phase separation column, electrospray ionization (ESI), negative mode, and single ion monitoring (SIM) with *m/z* 542 for TBBPA, 554 for $^{13}C_{12}$-TBBPA, 227 for BPA, 239 for $^{13}C_{12}$-BPA, 205 for OP, 219 for NP, 224 for internal standard of NP and OP[8], 265 for PCP, and 271 for $^{13}C_6$-PCP. Results show that the linearity of the calibration curves (1.0 - 5000 ng ml^{-1}) had correlation coefficients exceeding 0.999. The average recoveries of TBBPA and phenolic xeno-estrogens were above 85.0 % with correction using the added internal standard (Table 1). The quantitation limit in the air sample was 0.1 ng/m^3. The method enables the precise determination of TBBPA and xeno-estrogens using the added internal standard and was applied to the detection of trace amounts of TBBPA and xeno-estrogens in indoor air samples from apartment and house in Tokyo, Japan.

Indoor air was sampled from March to May 2003 in total 48 sampling points. These results was shown in Fig. 1.

Table 1 Recovery test

Spiked levels (ng/m^3)	TBBPA	BPA	NP	OP	PCP
10	99.5 (0.9)	100.4 (1.2)	101.2 (1.8)	101.0 (0.5)	100.6 (0.2)
100	87.0 (1.0)	101.9 (0.7)	99.0 (2.6)	96.4 (4.6)	100.3 (0.4)

N = 3

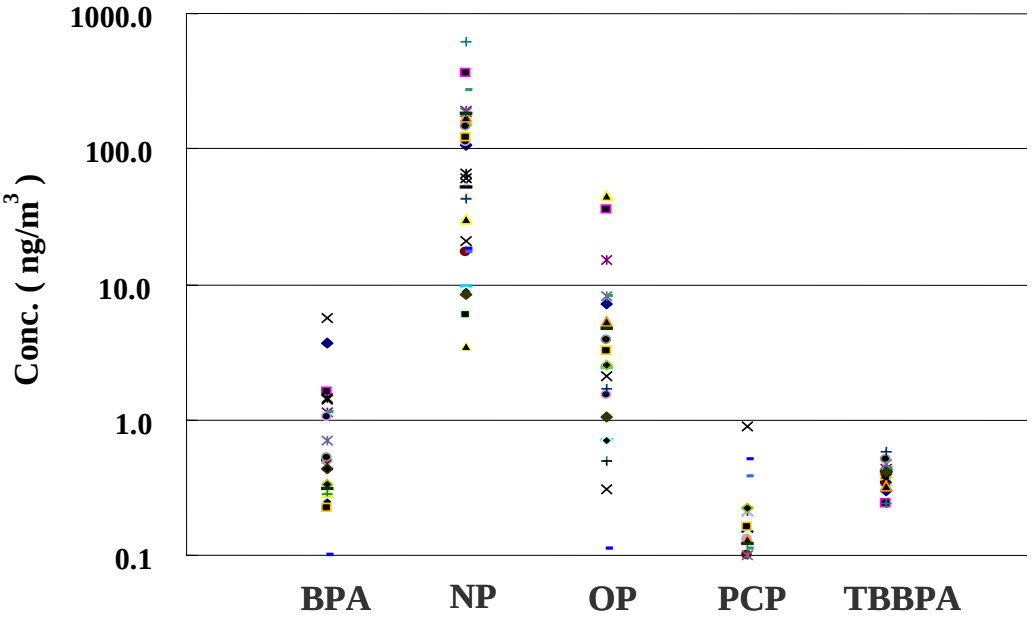

Fig. 1 TBBPA and phenolic xeno-estrogens in indoor air

Conclusions

Our findings suggest that the LC-MS method can detect the low levels of TBBPA and phenolic xeno-estrogens in air sample, and that indoor air pollution of these compounds was a trace level. However, NP was the most contamination in indoor than other compounds. Along with Japanese indoor air breathing data, average was used to calculate a daily these compounds intake for adult of 3 (TBBPA), 14 (BPA), 1611 (NP), 80 (OP) and 3 ng/day (PCP).

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