# CONTAMINATION LEVELS AND HEALTH ASSESSMENT OF TETRABROMOBISPHENOL A AND ITS RELATED COMPOUNDS IN INFANT FOODS

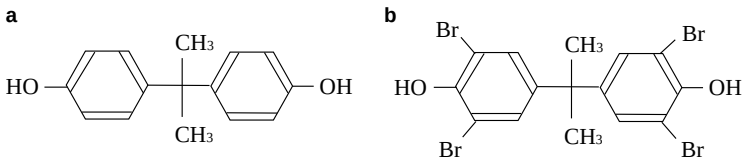
Teruyuki Nakao*, Hiromi Nishiki, Saori Nishida,Yasuhiro Iemura, Yuki Matsumoto, Mariko Ishizaka, Hideki Kakutani, Ema Akiyama, Souichi Ohta

Setsunan University: 45-1, Nagaotoge-cho, Hirakata, Osaka, Japan

## Introduction

Brominated flame retardants are well recognized as being highly effective flame retardants. Among them, Tetrabromobisphenol A (TBBPA, Fig. 1b) is the flame retardant with the largest production in the world today. TBBPA contributes to the fire safety of electrical and electric equipment and installations where printed wire boards are used, such as consumer electronics, office and communication equipment, automotive, aviation and all entertainment equipment. On the other hand, Bisphenol A (BPA, Fig. 1a) is well used as the feedstock of the plastic which is made from polycarbonate and epoxy resin. Their plastic and epoxy resin are used a lot of food container. In recent year, we have been interested in health effects of the infant by a various chemical substances. In fact, it was pointed out that the possibility of impairing intelligence growth of infant in our country[1)]. Further, Canadian government prohibited to sale and import of the BPA use feeding bottle in 2008. In 2010, they designated BPA as toxic substance. Comparing to BPA, it has not known the toxic mechanism of TBBPA yet. By the knowledge of *in vitro* and *in vivo* studies, it has been suggested the increase of the total cholesterol and the liver weight[2)]. It has been also reported that TBBPA passes a blood-placenta barrier easily[3)]. Moreover, TBBPA is concerned about the secondary toxicity by debrominated compounds of TBBPA (monobromo-, dibromo-, tribromobisphenol A) or BPA as final debrominated compounds. However, there is very little knowledge about contamination levels and health effect by the above compounds in the infant foods.

In this paper, we have cleared to contamination levels and daily intake of BPA, TBBPA and their related compounds, and assessed health effect; it was analyzed BPA, TBBPA and their related compounds in infant foods (vegetables, potatoes meats and powdered milks), and examined the toxicokinetic study in mice of TBBPA.




Bisphenol A (BPA) Tetrabromo bisphenol A (TBBPA)

**Fig. 1 Chemical structure of bisphenol A and tetrabromobisphenol A**

## Materials and methods

### *Samples collection*

Three samples for three different food categories (total 7 samples) were purchased from two grocery stores in Hirakata, Osaka, Japan. The collected samples included vegetables (pumpkin, tomato, pepper), tubers (potato) and meats (beef, chicken, pork). This food samples were washed with distilled water and measured weight, and then freeze-dried before analysis. The powdered milks were purchased from a baby product store in Yawata, Kyoto, Japan.

***Experimental method***

The analytical method for BPA and TBBPA measurement in infant foods (vegetables, tubers, meats and powdered milks) worked out according to manual by Ministry of the Environment of Japan. After spiking with $^{13}$C-labelled TBBPA and d-labelled BPA, food samples extracted for 30 min with methanol by ultrasonic wave. The extract was evaporated to dryness, allowing the gravimetric determination of the fat content. The extract was filtered by glass fiber filter, then the filtrate was extracted with ethyl acetate by liquid-liquid extraction. The extract was derivatized with diethyl sulfate. This solution concentrated to less than 1 ml, purification was performed Florisil column chromatography. The GC/HRMS detection was performed on a Hewlett-Packard 6890 gas chromatograph, equipped with a BPX-50 column (30 m x 0.25 mm, 0.25 μm film thickness), coupled to a JEOL JMS-700 high-resolution mass spectrometer. The mass filter operated at a resolution 10,000 in the selected ion-monitoring (SIM) mode using electronic impact as the ionization technique.

**Results and discussion**

As shown in Table 1, the levels of BPA and TBBPA were investigated in vegetables and potato. As a result, both compounds were detected in vegetables and potato, but the either of the contamination level is very low, showing below 1.0 ng/g, wet weight basis.

Table 1 Contamination levels of TBBPA and BPA (ng/g) in vegetables and tuber

| | Pumpkin | Tomato | Pepper | Potato |
|---|---|---|---|---|
| TBBPA | 0.71 | 0.37 | 0.43 | 0.037 |
| BPA | 0.81 | 0.57 | 0.094 | 0.051 |

The concentrations of BPA and TBBPA in the meats (chicken, pork and beef) were presented in Table 2. Levels of TBBPA and BPA were 2.2 - 3.9 ng/g, and 2.9 - 4.1 ng/g, respectively. From these results, it was observed a small difference among this meat samples. Consequently, we assumed that the contamination levels of the infant foods with TBBPA and BPA were minor contamination.

As shown in Table 3, TBBPA and BPA were detected in powdered milk. The contamination levels of TBBPA were ranged from 3.3 to 3.8 ng/g and a difference was not observed. With respect to BPA, it was ranged from

3.5 to 11 ng/g, that of Company C was higher. To clarify the reason, it is presently investigating the milk of Company C by using products of different lot number.

Table 2 Contamination levels of TBBPA and BPA (ng/g) in meats

| | Chicken | Pork | Beef |
|---|---|---|---|
| TBBPA | 2.2 | 3.5 | 3.9 |
| BPA | 4.1 | 3.9 | 2.7 |

Table 3 Contamination levels of TBBPA and BPA (ng/g) in powdered milk

| | Company A | Company B | Company C | |
|---|---|---|---|---|
| | Powdered milk (from 0 month) | Powdered milk (from 0 month) | Powdered milk (from 0 month) | Powdered milk (from 9 month) |
| TBBPA | 3.5 | 3.5 | 3.3 | 3.8 |
| BPA | 3.5 | 3.9 | 11 | 4.2 |

Next, by in vivo study by use of C57BL/6 mice, we examined to toxicokinetics of TBBPA. Confirming excretion and distribution after 24 hrs, about 46% of oral dose was excreted in feces and urine. The most of the parent TBBPA as administrated substance was excreted in feces. Then, we also tried to analyze TBBPA glucuronate conjugate and its sulfate conjugate. In feces, TBBPA conjugate occupied 21%. From these result, the sum of parent and conjugation were higher than 60% in feces. It was cleared that the main excretion route of TBBPA was the feces.

Table 4 Excretion ratio (%) of TBBPA in mice after oral administration

| | Feces | Urine |
|---|---|---|
| TBBPA | 44 | 2.0 |
| TBBPA conjugate | 21 | 0 |

Then, we investigated to distribution of the internal organs with parent TBBPA. The distribution of each internal organ to the total oral dose was very low. Fig. 2 shows level of each internal organ of TBBPA after oral administration. The distribution ratio was the highest in the liver. Next, it was the order of being intestine, heart, spleen, kidney, intestinal membrane fat and brown fat. It was cleared that TBBPA hardly accumulate to high-

fat-containing organ in C57BL/6 mice, TBBPA excrete in feces immediately. It's interesting to note that the glucuronic acid conjugation of TBBPA was detected in the urine, and debromination of TBBPA was detected in the liver. It has been reported that TBBPA and its debrominated compounds (TriBBPA, DiBBPA and MoBBPA) have estrogen-like activity in vitro system[4]; These above compounds competed with 17β-estradiol for binding to the estrogen receptor in MCF-7 cells, and the binding potential decreased with increasing number of bromo-substitutions.

Further study is needed to examine the health effect with these debrominated compounds.

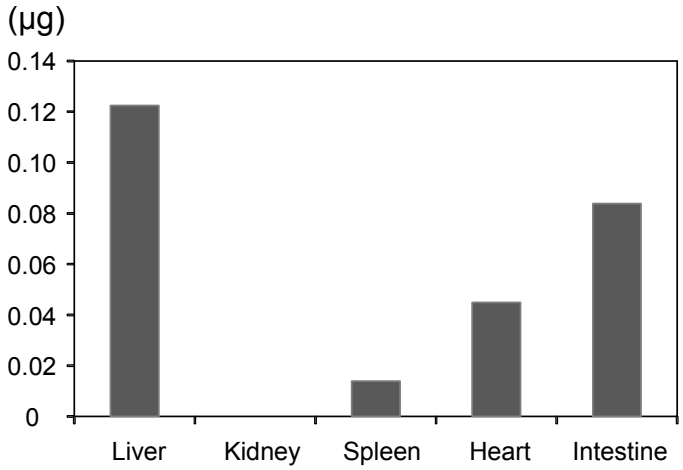



Fig. 2 Organ distribution of TBBPA in mice after oral administration

**Acknowledgements**

This work was supported by the Health and Labour Sciences Research Grant (H23-Food-Young scientist-017).

**References:**

1. Casba L, Tiber H, Klara SB, Jeremy B, Neil JM. (2008) *Proc Natl Acad Sci USA* 105: 14187-14191

2. Tada Y, Fujitani T, Yano N, Takahashi H, Yuzawa K, Ando H, Kubo Y, Nagasawa A, Ogata A, Kamimura H. (2006) *Food Chem Toxicol*. 44(8): 1408-13

3. Fujitani T, Tada Y, Takahashi H, Yano N, Ando H, Kubo Y, Yuzawa K, Nagasawa A, Ogata A, Kamimura H. (2006) *Ann. Rep. Tokyo Metr. Inst. P. H.* 57: 361-365

4. Samuelsen M, Olsen C, Holme JA, Meussen-Elholm E, Bergmann A, Hongslo JK. (2001) *Cell Biology and Toxicology* 17: 139-151