

INTAKE ASSESSMENT OF DECHLORANE PLUS AND POLYBROMINATED DIPHENYL ETHERS VIA INHALATION AND DIET IN JAPAN

Kakimoto K^{1*}, Nagayoshi H¹, Takagi S¹, Akutsu K¹, Konishi Y¹, Kajimura K¹, Toriba A², Hayakawa K²

¹ Osaka Prefectural Institute of Public Health, 1-3-69, Nakamichi, Higashinari-ku, Osaka 537-0025, Japan;

² Institute of Medical, Pharmaceutical and Health Sciences, Kanazawa University, Kakuma-machi, Kanazawa, Ishikawa 920-1192, Japan

Introduction

In recent years, there has been increasing concern regarding worldwide pollution by halogenated flame retardants. In 2009, penta-BDE and octa-BDE were included in the Conference of the Parties (COP-1) of the 4th Stockholm Convention on persistent organic pollutants (POPs) (www.pops.int). Recent reports indicated that deca-BDE accounted for a substantial proportion of total PBDE congeners, irrespective of whether it was in the environmental or biological matrix.

Dechlorane Plus (DP) is a highly chlorinated additive flame retardant, and has a cost advantage over comparable brominated flame retardants such as PBDE. DP has been manufactured for more than 40 years, and is used in electrical wire and cable coatings, computer connectors, and plastic roofing materials¹. DP has typical characteristics of a persistent organic pollutant, such as high chlorine content, and very high octanol/water partition coefficient. There have been some studies on toxicity of DP in the effects of cytotoxicity, mRNA expression², and blood-brain barrier permeability of anti-DP in fish³. The detection of DP has recently increased in East Asia^{4,5,6,7,8}. Japan's geographical location makes it susceptible to influence by air or ocean currents from the Asian continent. At the 20th Symposium on Environmental Chemistry, held in Japan, Sakiyama and Nakano reported the presence of DP in soil and dust samples from Japan⁹. However, no data are available on the level of exposure to DP in Japan. The primary routes for non-occupational exposure to environmental pollutants are dietary and inhalation pathways.

The purpose of the present study is to determine total DP exposure via atmosphere and diet in Japan. In addition to DP, we determined the levels of PBDE for a comprehensive assessment of flame retardant contamination.

Materials and methods

1. *Sample collection*

1.1 *Atmospheric particle samples*

Samples were collected in the vicinity of a research laboratory located in the eastern urban area of Osaka City, Japan (latitude 34°68'N, longitude 135°535'E). Atmospheric samples were collected using a high-volume air sampler equipped with a glass-fiber filter (GFF) at a flow rate of 0.5 m³ min⁻¹. Sampling periods were November to December 2012, and February to March 2013. The GFF was exchanged every week. After sampling, the filters were packaged with aluminum foil and stored in a freezer until analysis.

1.2 *Market basket study*

The market basket study samples were prepared on the basis of official food classification and consumption data from the National Nutrition Survey, conducted by the Ministry of Health and Welfare of Japan. A total of 123 food samples were purchased from supermarkets in Osaka in 2012. These food samples were cooked or prepared for consumption in a typical manner and were then weighed according to the daily consumption amounts. The weighed foods were mixed and homogenized to form 13 food-group composites numbered I to XIII, as listed in Table. The homogenized samples were then frozen and stored until use.

The 13 food groups used in the market basket study, Osaka 2012

Group No.	Composition (representative example)	No. of food varieties	Lipid content (%)	Daily intake per capita (g day ⁻¹)
I	Rice and rice products (rice, rice powder)	2	0.04	334
II	Grains, seeds, and tubers (wheat, wheat products, potato, potato products, sesame)	15	1.8	176
III	Sugar and confectionary (doughnut, potato chips, biscuit)	8	11.9	32
IV	Oils and fats (butter, margarine, salad oil, lard)	4	93	10
V	Legumes and their products (soy bean, tofu, fried bean curd, natto, chickpea)	7	9.2	52
VI	Fruits (strawberry, orange, banana, apple, pineapple, kiwi fruit)	11	0.2	107
VII	Brightly colored vegetables (tomato, carrot, spinach, pumpkin, broccoli)	10	0.1	95
VIII	Other vegetables, mushrooms, and seaweeds (cucumber, white radish, eggplant, dried barilla)	13	0.03	184
IX	Beverages (soft drink, beer, coffee, distilled spirit)	7	0.03	679
X	Fish, shellfish, and their products (Spanish mackerel, salmon, fish sausages, canned tuna)	17	6.6	72
XI	Meat and eggs (beef, pork, chicken, chicken egg)	13	13.3	120
XII	Milk and dairy products (milk, cheese, yoghurt, ice cream)	5	6.2	111
XIII	Seasonings and other processed foods (soy sauce, mayonnaise, ketchup, curry block, vinegar)	11	7.2	101

2. Chemicals

Non-labeled DP (*syn*- and *anti*-) and PBDE, ¹³C₁₂-labeled PBDE mixture (BDE-28, 47, 99, 154, 153, 183, 197, 207, 209) were purchased from Wellington Laboratories (Ontario, Canada). The ¹³C₁₀-labeled DP (*syn*- and *anti*-) standards were purchased from Cambridge Isotope Laboratories (MA, USA). Organic solvents of pesticide analysis grade were used for the extraction and cleanup of samples, and sulfuric acid-impregnated silica gel of dioxin analysis grade was purchased from Wako Pure Chemicals (Osaka, Japan).

3. Extraction and cleanup

3.1 Atmospheric particle samples

The filters were extracted ultrasonically for 30 min with 50 mL of dichloromethane containing ¹³C-labeled standard mixtures (¹³C₁₀-labeled DP, (*syn*- and *anti*-), 0.25 ng each; ¹³C₁₂-labeled PBDE, 0.25–1.25 ng). After centrifugation, the supernatant was concentrated to dryness and re-dissolved in hexane. The solution was cleaned-up using a 44% sulfuric acid-impregnated silica gel column (1 g). Hexane (10 ml) was used as an eluent. After adding 20 µl of nonane, the eluate was evaporated until hexane was completely removed. Finally, 1 µl of sample solution was used for the analyses of DP and PBDE, respectively, via gas chromatography-mass spectrometry (GC/MS).

3.2 Market basket study

Samples (10 g) were spiked with ¹³C-labeled standard mixtures. The samples were extracted using a mixture of n-hexane and diethyl ether (2:1, v/v) twice via a homogenizer and then followed by centrifugation. The organic layer was collected and washed with 2% NaCl. After dehydration with Na₂SO₄, the organic solvent was concentrated using a rotary evaporator and the residue was re-dissolved in an acetone–cyclohexane mixture (3:7, v/v). Lipid purification was then performed using gel permeation chromatography. The column (CLNpak EV-G AC, 100 mm, 20 mm i.d., CLNpak EV-2000 AC, 300 mm, 20 mm i.d., Showa Denko, Tokyo, Japan) was eluted with an acetone–cyclohexane mixture (3:7, v/v) at 40 °C. The flow rate was 5 ml min⁻¹; the first 60 ml of the eluate was discarded to remove the bulk of the lipids, and the subsequent 100 ml of the eluate was collected. The eluate was evaporated to dryness and re-dissolved in hexane. The solution was applied to a 44% sulfuric acid-impregnated silica gel column. The subsequent treatment was the same as that for filter samples.

4. GC/MS conditions

GC/MS analyses were performed using a gas chromatograph coupled to a high-resolution mass spectrometer (JMS-800D, JOEL, Tokyo, Japan). The GC conditions were as follows: column, capillary non-polar column (DB-1, 15 m, 0.25 mm i.d., 0.1 μm film thickness, J&W Scientific, CA, USA); column temperature program, 100 $^{\circ}\text{C}$ (held for 2 min) to 330 $^{\circ}\text{C}$ (held for 5 min) at 10 $^{\circ}\text{C min}^{-1}$; carrier gas, helium; column head pressure, 10 psi; injection temperature, 250 $^{\circ}\text{C}$; injection mode, splitless (splitless time, 1.5 min). All target DP and PBDE were eluted from the column within 30 min under these conditions. The MS conditions were as follows: ionization mode, electron ionization mode; electron energy, 38 eV; filament current, 500 μA ; ion source temperature, 270 $^{\circ}\text{C}$; resolution, 10,000 (10% valley definition). The DP and PBDE were quantified by the isotope dilution method using the corresponding ^{13}C -labeled congeners.

Results and discussion

1. Atmospheric particle samples

DP was detected in all samples and ΣDP concentrations ranged from 7.1 to 15.4 pg m^{-3} . The mean concentration of ΣDP in this study (11 pg m^{-3}) is lower than that reported for urban sites in China (15.6 pg m^{-3})¹⁰ and the Great Lakes area near a DP manufacturing point (34 pg m^{-3})¹¹; and is higher than concentrations recorded at rural sites in China (3.5 pg m^{-3})¹⁰ and in the Shanghai area (5.48 pg m^{-3})¹². The fractional abundance of the *anti*-isomer (f_{anti}) is calculated by dividing the concentration of *anti*-DP by the sum of the *syn*- and *anti*-DP concentrations. The mean f_{anti} value in this study was 0.74 ± 0.05 (mean \pm one standard deviation). The f_{anti} value of commercial DP products from the Great lakes area differed between studies, ranging from 0.65 to 0.80^{1,11,13}. It is reported that the stereo-selective depletion of *anti*-isomer was caused by UV radiation from sunlight during atmospheric transport¹⁴. The f_{anti} values of DP in the filter samples in this study were similar to those of commercial products, so we considered that atmospheric DP pollution originated mainly from DP-containing products used in Japan.

PBDE was detected in all filter samples, at concentrations between 9.9 and 23.3 pg m^{-3} for ΣPBDE . Among the PBDE congeners, deca-BDE was the most abundant in the atmosphere in this study. One reason for the high concentrations of deca-BDE is that technical deca-BDE has not yet been banned, and remains one of the most widely used forms of BFR; another reason is that only particulate samples were analyzed in this study, whereas low-brominated BDEs exist both in gas- and particle phases. Strandberg et al. reported that the percentages present in the gas phase were about 80% for BDE-47, about 55–65% for BDE-100 and -99, and about 30% for BDE-154 and -153¹⁵. Nona-BDE and deca-BDE were reported to occur almost exclusively in particle-phase samples, irrespective of the sampling season, and deca-BDE (BDE-209) was detected at relatively high concentrations compared to other PBDE congeners¹⁶. We considered BDE-209 as a reasonably representative indicator of atmospheric PBDE contamination in this study.

2. Market basket study

DP was detected in four food groups at ΣDP concentrations of 1.5–3.3 pg g^{-1} wet wt. PBDE was detected in six food groups at ΣPBDE concentrations of 74.6–308 pg g^{-1} wet wt. Both DP and PBDE were detected in groups III, V, X, and XI; and PBDE alone was detected in IV and XIII. Groups IV and XIII contained oil products of animal and plant origin. Total PBDE concentration was highest in the fish group (group X) and BDE-47 was highest among the congeners. With the exception of the fish group, BDE-209 was most abundant. A recent dietary study also indicated that BDE-209 accounts for a large proportion of PBDE in food groups except for fish^{17,18}.

The mean f_{anti} value was 0.62 ± 0.09 (mean \pm one standard deviation), which is less than that of atmospheric samples in this study. Some reports indicate that *syn*-DP is more bio-accumulative than *anti*-DP, and tends towards higher trophic levels^{19,20,21}, thereby explaining the higher *syn*-DP ratio Group X (fish, shellfish, and their products).

3. DP and PBDE intake estimation

We estimated the mean daily intakes of ΣDP and ΣPBDE from atmosphere were found to be 174 pg day^{-1} and 238 pg day^{-1} , respectively; when we set the mean inhalation rates as 15.7 $\text{m}^3 \text{day}^{-1}$ (US EPA, 2011) for an adult

human. The dietary intakes of Σ DP and Σ PBDE by an average Japanese adult were calculated to be 576 pg day^{-1} and 61.8 ng day^{-1} , respectively (non-detected values were treated as zero). These results indicate that DP exposure via inhalation is one-third of that via diet, and that PBDE exposure occurs mainly via diet. The atmospheric exposure to DP was around three-quarters that of PBDE, and dietary exposure to DP was around one percent of that for PBDE.

References

1. Tomy, G.T., Pleskach, K., Ismail, N., Whittle, D.M., Helm, P.A., Sverko, E., Zaruk, D., Marvin, C.H. (2007); *Environ Sci Technol.* 41: 2249–2254
2. Crump, D., Chiu, S., Gauthier, L.T., Hickey, N.J., Letcher, R.J., Kennedy, S.W. (2011); *Comp Biochem Physiol C: Toxicol Pharmacol.* 154: 129–134.
3. Zhang, Y., Wu, J.P., Luo, X.J., Wang, J., Chen, S.J., Mai, B.X. (2011); *Environ Pollut.* 159: 3647–3652
4. Kang, J.H., Kim, J.C., Jin, G.Z., Park, H., Baek, S.Y., Chang, Y.S. (2010); *Chemosphere.* 79: 850–854
5. Qi, H., Liu, L., Jia, H., Li, Y.F., Ren, N.Q., You, H., Shi, X., Fan, L., Ding, Y. (2010); *Environ Sci Technol.* 44: 2305–2308
6. Qiu, X., Zhu, T., Hu, J. (2010); *Chemosphere.* 80: 1207–1212
7. Ma, W.L., Liu, L.Y., Qi, H., Sun, D.Z., Shen, J.M., Wang, D.G., Li, Y.F. (2011); *Environ Int.* 37: 66–70
8. Zhao, Z., Zhong, G., Moller, A., Xie, Z., Sturm, R., Ebinghaus, R., Tang, J., Zhang, G. (2011); *Chemosphere.* 83: 984–990
9. Sakiyama, T., Nakano, T. (2011); The proceedings of 20th symposium on environmental chemistry held by Japan Society for Environmental Chemistry. (in Japanese)
<http://www.j-ec.or.jp/conference/20th/20_program.pdf>, accessed 05th April, 2013.
10. Ren, N., Sverko, E., Li, Y.F., Zhang, Z., Harner, T., Wang, D., Wan, X., McCarty, B.E. (2008); *Environ Sci Technol.* 42: 6476–6480
11. Hoh, E., Zhu, L., Hites, R.A. (2006); *Environ Sci Technol.* 40: 1184–1189
12. Yu, Z., Liao, R., Li, H., Mo, L., Zeng, X., Sheng, G., Fu, J. (2011); *Environ Pollut.* 159: 2982–2988
13. Sverko, E., Tomy, G.T., Reiner, E.J., Li, Y.F., McCarty, B.E., Arnot, J.A., Law, R.J., Hites, R.A. (2011); *Environ Sci Technol.* 45: 5088–5098
14. Moller, A., Xie, Z., Sturm, R., Ebinghaus, R. (2010); *Environ Sci Technol.* 44: 8977–8982
15. Strandberg, B., Dodder, N.G., Basu, I., Hites, R.A. (2001); *Environ Sci Technol.* 35: 1078–1083
16. Hayakawa, K., Takatsuki, H., Watanabe, I., Sakai, S. (2004); *Chemosphere.* 57: 343–356
17. UK Food Standards Agency. (2006);
<<http://www.food.gov.uk/science/surveillance/fsisbranch2006/fsis1006>>, accessed 05th April, 2013.
18. Chen, M.L., Wang, L., Chi, Y.K., Mao, C.C., Lung, S.C., Mao, I.F. (2012); *Sci Total Environ.* 431: 183–187
19. Wu, J.P., Zhang, Y., Luo, X.J., Wang, J., Chen, S.J., Guan, Y.T., Mai, B.X. (2010); *Environ Sci Technol.* 44: 606–611
20. Xian, Q., Siddique, S., Li, T., Feng, Y.L., Takser, L., Zhu, J. (2011); *Environ Int.* 37: 1273–1284
21. She, Y.Z., Wu, J.P., Zhang, Y., Peng, Y., Mo, L., Luo, X.J., Mai, B.X. (2013); *Environ Pollut.* 174: 164–170