Dissertation

Findings on human exposure of halogenated organic pollutants through food and atmosphere

食品及び大気を介した有機ハロゲン汚染化学物質によるヒト曝露実態の解明

Graduate School of
Medical Sciences
Kanazawa University

Division: Pharmaceutical Sciences

Laboratory: Hygienic Chemistry

School registration No.：1329012007

Name: Kensaku Kakimoto

Primary supervisor name: Akira Toriba

Contents

ABBREVIATIONS USED

AhR, aryl hydrocarbon receptor

Arnt, AhR nuclear translocator

ClPAHs, chlorinated polycyclic aromatic hydrocarbons

ClPyr, 1-chloropyrene

COSY, correlated spectroscopy

DP, dechlorane plus

EC_{50}, half maximal effective concentration

FL, fluorescence detector

HBCD, hexabromocyclododecane

HMBC, heteronuclear multiple bond correlation

HSQC, heteronuclear single quantum coherence

LC, liquid chromatography

LC-MS/MS, LC-tandem mass spectrometry

NMR, nuclear magnetic resonance

PAHs, polycyclic aromatic hydrocarbons

PBDEs, polybrominated diphenyl ethers

PCDDs, polychlorinated dibenzo-*p*-dioxins

PCDFs, polychlorinated dibenzofurans

PDA, photodiode array detector

POPs, persistent organic pollutants

PM, particulate matter

P450, cytochrome P450

SRM, selected reaction monitoring

TSP, total suspended particulate matter

1. General introduction and aim

In 2015, the world's population is over 7.3 billion and expected to rise to 9.5 billion by 2050. The growing population, coupled with rapid economic expansion and consumerism, has given rise to increasing environmental pollution across the world. In Japan, there have been serious environmental and food pollution incidents caused by organic pollutants, such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and polybrominated diphenyl ethers (PBDEs). These compounds are classified into two general groups, those generated intentionally and those not intentionally. As is well known, PCBs and PBDEs are intentionally generated compounds, the former used in insulating oils, plasticizers, and paints, and the latter in flame retardants. On the other hand, PAHs, PCDDs, and PCDFs are generated unintentionally occurring in combustion gas or existing as impurities in chemical products. Contaminants that accumulate in the environment and in living organisms have been designated as persistent organic pollutants (POPs) by the Conference of the Parties of the Stockholm Convention on POPs (www.pops.int) and the production and usage of them are restricted in treaty countries (Fig. 1).

In my previous study, I investigated the food contamination and human exposure of

hexabromocyclododecane (HBCD) (Fig. 2), which is an additive brominated flame retardant (BFR) and used in expanded and extruded polystyrene (EPS/XPS) for insulation, in high impact polystyrene (HIPS) for electronics, in textiles, and in polyvinyl chloride (PVC) for cable sheathings. In Japan HBCD has been used since the mid-1980s. Its technical demand was around 2,577 metric tons in 2009, 84% for polystyrene foam, 15% for textiles, and 1% for other uses (Ministry of Economy, Trade and Industry of Japan (Fig. 3)). The European Union (EU) published a risk assessment report on HBCD in 2008 (EU, 2008). The report described how HBCD disrupts the thyroid hormone systems, causing memory defects and significant changes in spontaneous behavior and learning (EU, 2008). In the mid-2000s it was felt there was insufficient information about HBCD pollution in Japan, so contamination was investigated through the determination of its concentration in food (market basket study; Kakimoto et al., 2012, fish oil supplements; Kakimoto et al., 2008a) and human breast milk (Kakimoto et al., 2008b). For breast milk samples, we showed that the time trend of HBCD concentrations appeared to be related to that of the technical HBCD demands level (Fig. 3&4) with the elevation in ΣHBCD concentration appearing to be later than the elevation in concentration of ΣPBDEs. The concentration of ΣHBCD in the samples obtained in 2000 or later was in the range 1.0–4.0 ng g^{-1} lipid weight, and these levels

were approximately the same as those of PBDEs (ΣPBDEs; #28, #37, #75, #47, #66, #77, #100, #99, #85, #154, #153, #138, and #183) in human breast milk (1.39 ng·g^{-1} lipid weight) (Akutsu et al., 2003). In 2007 the estimated average level of HBCD intake by breast-feeding infants (aged 0 days to 3 months) was 7.5 ng kg^{-1} body weight day^{-1} in breast milk samples collected from women aged 25–29. This value was derived using a mathematical formula in the EU risk assessment report (2008) (Fig. 5). The representative chromatograms of HBCD in breast milk sample are shown in Fig. 6. For the market basket study samples we detected HBCD in fish group in 14 food groups (Table 1) and the estimated the dietary intake of HBCD was 6 ng kg^{-1} body weight day^{-1}(Kakimoto et al., 2008b; Kakimoto et al., 2012). We also detected HBCD in fish oil supplements (Table 2) and estimated the daily intake of ΣHBCD according to the daily doses proposed by the product manufacturers, as shown in Table 3. The maximum intake estimated in this study (200 ng day^{-1}) was higher than the reported median intake of HBCD (141 ng day^{-1}) (Covaci et al., 2006). Our data indicate that the frequent consumption of oil supplements will increase dietary exposure to HBCD (Kakimoto et al., 2008a). The HBCD levels in both breast milk and food products were much lower than the no-observed-adverse-effect-level (NOAEL) of 10.2 mg kg^{-1} body weight day^{-1} for HBCD (Ema et al., 2008). The various reports revealed the bioaccumulation

potential, toxicity, and persistent character of HBCD, and there was enough circumstantial evidence to regard HBCD as a hazardous compound. The industry began to refrain from using HBCD step by step, and finally HBCD was designated as a new persistent organic pollutant (Annex A) in COP6 of the Stockholm Convention held in 2013. Our work was cited in the reports of POPRC6 (2010) which made a recommendation to add HBCD to the list of POPs in Annex A.

Although twenty-three compounds are now designated as POPs, unrestricted, persistent, bio-accumulative, and toxic hazardous environmental pollutants still exist. Many of these have not been adequately assessed and the shortage of information about the actual pollution conditions is a serious problem. In addition, POPs' legacy still remains in the environment (Tue et al., 2013) and the biosphere (Glynn et al., 2011; Tornkvist et al., 2011). It is therefore also important to monitor these compounds for the assessment of the effectiveness of the Stockholm Convention.

In this study, the halogenated POPs as previously described (PBDE and HBCD), and the potentially hazardous compounds Dechlorane Plus and chlorinated polycyclic aromatic hydrocarbons were particularized. Dechlorane Plus (DP, synonyms: DechPlus or Dechlorane 605, CAS number: 13560-89-9, IUPAC name: 1,2,3,4,7,8,9,10,13,13,14,14-dodecachloro-1,4,4a,5,6,6a,7,10,10a,11,12,12a-dodecahydr

o-1,4,7,10-dimethanodibenzo[a,e]cyclooctene) is an industrially manufactured chemical used as a flame retardant. Chlorinated polycyclic aromatic hydrocarbons (ClPAHs) (Fig 7& 8) are unintentionally generated compounds. These compounds are still unrestricted and there is currently little information about their environmental pollution levels or toxic effects. It is therefore important to investigate the pollution and exposure level of these compounds. In this thesis, DP pollution in food and the atmosphere (sections *2.1.* to *2.3.*), atmospheric ClPAHs (section *2.4.*), the human metabolic pathway of ClPAHs (section *2.5.*), and the particle size distributions of ClPAHs (section *2.6.*) are described.

Table 1 Information of 14 food groups of the market basket study in Osaka, 2007

Food group	Composition	No. of food variety	Lipid content (%)	Daily intake per capita (g day^{-1})	HBCD (ng g^{-1} wet weight)
Ⅰ	Rice and rice products	2	0.2	341	N.D.
Ⅱ	Grains, seeds, and tubers	15	1.5	174	N.D.
Ⅲ	Sugar and confectioneries	6	9.4	35	N.D.
Ⅳ	Oils and fats	4	93	11	N.D.
Ⅴ	Legumes and their products	6	6	58	N.D.
Ⅵ	Fruits	12	0.2	121	N.D.
Ⅶ	Brightly colored vegetables	13	0.3	93	N.D.
Ⅷ	Other vegetables, mushrooms and seaweeds	13	0.3	184	N.D.
Ⅸ	Beverages	7	0	616	N.D.
Ⅹ	Fish,shellfish, and their products	23	7.7	82	3.6 (α1.5, β0.03, γ2.1)
ⅩⅠ	Meat and eggs	8	18	121	N.D.
ⅩⅡ	Milk and dairy products	5	6.7	143	N.D.
ⅩⅢ	Seasonings and other processed foods	11	8.8	93	N.D.
ⅩⅣ	Drinking water	1	0	250	N.D.

Detection limit 0.007 ng/g.
Abbreviations: N.D., not detected.

Table2 Sample information and concentrations of HBCDs and PBDEs (ng g^{-1} lipid wt.)

sample No.	sample type (sample site)	recommended dose (g/day)	conc. HBCD (ng/g lipid weight)				conc. Σ10PBDEs (ng/g lipid weight)[c]
			α	β	γ	Σα,β, and γ	
1	sardine oil (Japan)*	1-3	43	1.1	23	67	6.0
2	lamprey oil	0.6-1.8	4.5	1.8	3.0	9.3	1.8
3	lamprey oil	1.5-3	2.1	<0.2	<0.5	2.1	7.8
4	globe fish liver oil	0.75	9.5	<0.2	1.5	11	3.6
5a	cod liver oil (the North sea)*	1-2	3.7	0.4	1.3	5.4	32
5b	cod liver oil (the North sea)*	1-2	5.0	0.3	0.9	6.2	28
6	cod liver oil (the North sea)*	0.4-1	4.0	<0.2	<0.5	4.0	16
7a	shark liver oil (Japan)*	0.75-1.75	22	0.7	21	44	49
7b	shark liver oil (Japan)*	0.75-1.75	24	<0.2	21	45	53
8	shark liver oil (Japan)*	0.75-1.75	25	0.3	19	44	52
9	shark liver oil (New Zealand)*	1.8	<0.2	<0.2	<0.5	n.d.	0.7
10	shark liver oil (New Zealand)*	0.6-1.2	<0.2	<0.2	<0.5	n.d.	0.2
11	shark liver oil (New Zealand)*	1.5	<0.2	<0.2	<0.5	n.d.	0.6
12	shark liver oil	2.6	<0.2	<0.2	<0.5	n.d.	0.1
13	shark liver oil	0.5	4.0	1.4	1.9	7.3	15
14	shark liver oil	0.6-1.5	<0.2	<0.2	<0.5	n.d.	0.3
15	shark liver oil	0.9-1.2	<0.2	<0.2	<0.5	n.d.	0.1
16	shark liver oil	1.8-2.7	<0.2	<0.2	<0.5	n.d.	0.5
17	shark liver oil	2.2-2.8	0.4	<0.2	<0.5	0.4	0.1
18	seal oil (Canada)	1.5	0.4	<0.2	<0.5	0.4	0.9
19	seal oil (Canada)	0.9	0.5	<0.2	<0.5	0.5	0.8
20	sea snake oil	0.9-1.8	2.1	1.3	1.7	5.1	4.7

[c]Σ10PBDEs: #28, #47, #49, #66, #99, #100, #153, #154, #155, and #183 (Akutsu et al., 2006)

*no heating step in the refining process

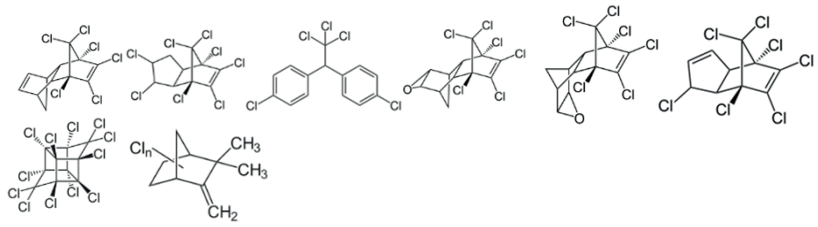
Table3 Estimated daily intake (ng day^{-1}) of HBCD isomers from oil supplements

sample No.	ΣHBCD (α,β, and γ)
1	67-200
2	5.6-16.7
3	3.2-6.3
4	8.3
5a	5.4-10.8
5b	6.2-12.4
6	1.6-4.0
7a	33-77
7b	34-79
8	33-77
9	1.6
10	0.5-1.1
11	1.4
12	2.3
13	3.7
14	0.5-1.4
15	0.8-1.1
16	1.6-2.4
17	0.9-1.1
18	0.6
19	0.5
20	4.6-9.2

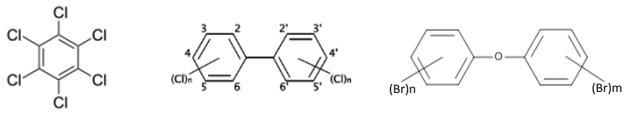
Table 4 DP pollution near production facilities / e-waste site

Air	26,000 pg m^{-3} *(China) Wang et al. 2010* 490 pg m^{-3} *(US) Hoh et al.2006*
Indoor dust	21,000 ng g^{-1} d.w. *(e-waste) Wang et al. 2011* 5,680 ng g^{-1} d.w. *(US) Zhu et al. 2007*
Sediment	300 ng g^{-1} d.w. *(US) Qiu et al.2007*
Soil	13,000 ng g^{-1} d.w. *(China) Wang et al. 2010*
Fish	9,600 ng g^{-1} l.w. *(e-waste) Wu et al. 2010*
Human Blood	2,900 ng g^{-1} l.w. *(China) Zhang et al. 2013*

•**Pesticides**: aldrin, chlordane, DDT, dieldrin, endrin, heptachlor, hexachlorobenzene, mirex, toxaphene;



•**Industrial chemicals**: hexachlorobenzene, polychlorinated biphenyls (PCBs), polybrominated biphenyl ethers (PBDE)



•**By-products**: hexachlorobenzene; polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans (PCDD/PCDF), and PCBs.

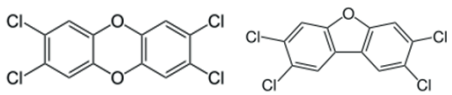


Fig.1 Chemical structure of legacy POPs

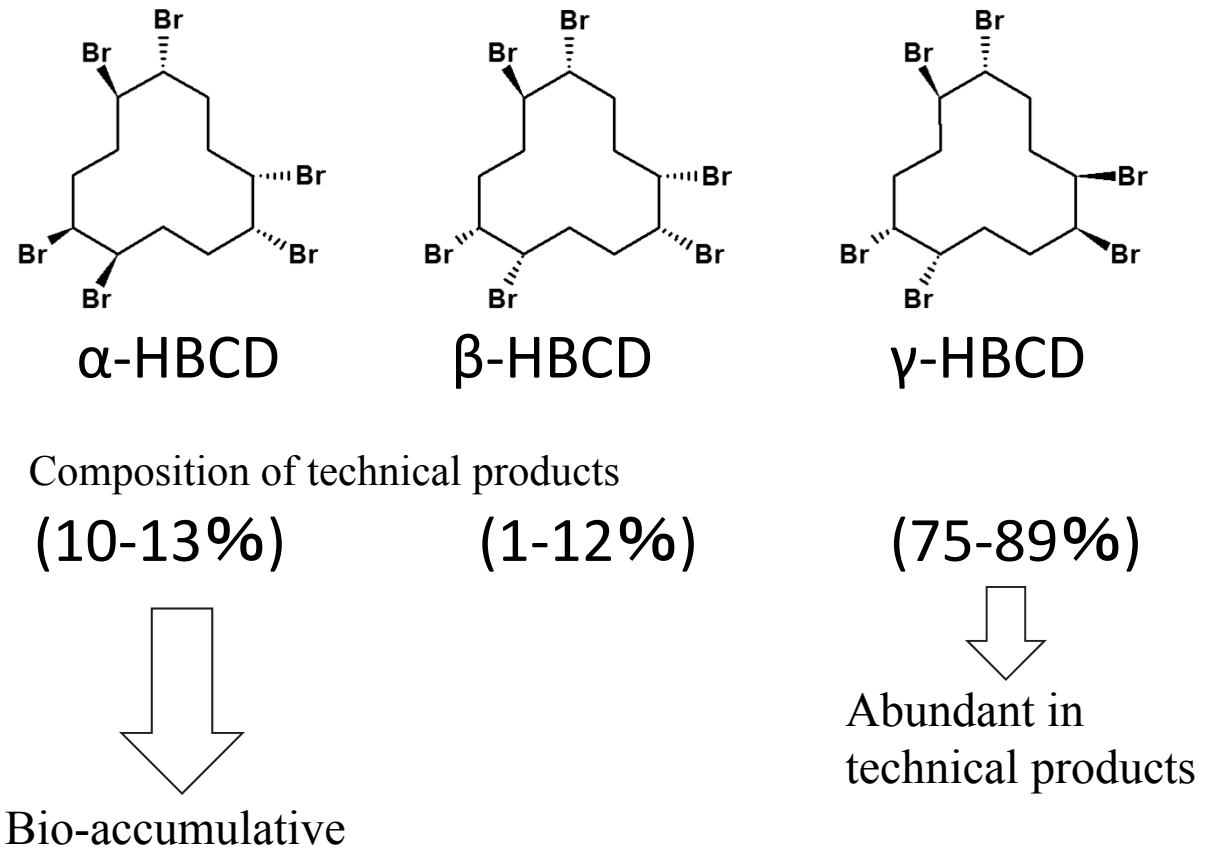


Fig.2 Chemical structure of HBCD

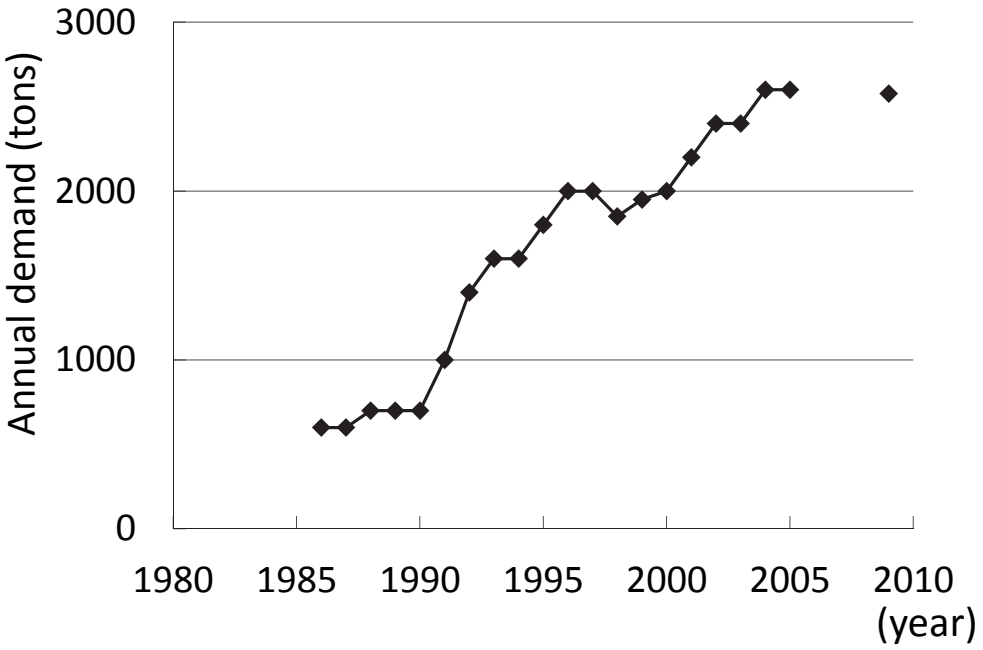


Fig.3 Trend in the annual demand of HBCD in Japan

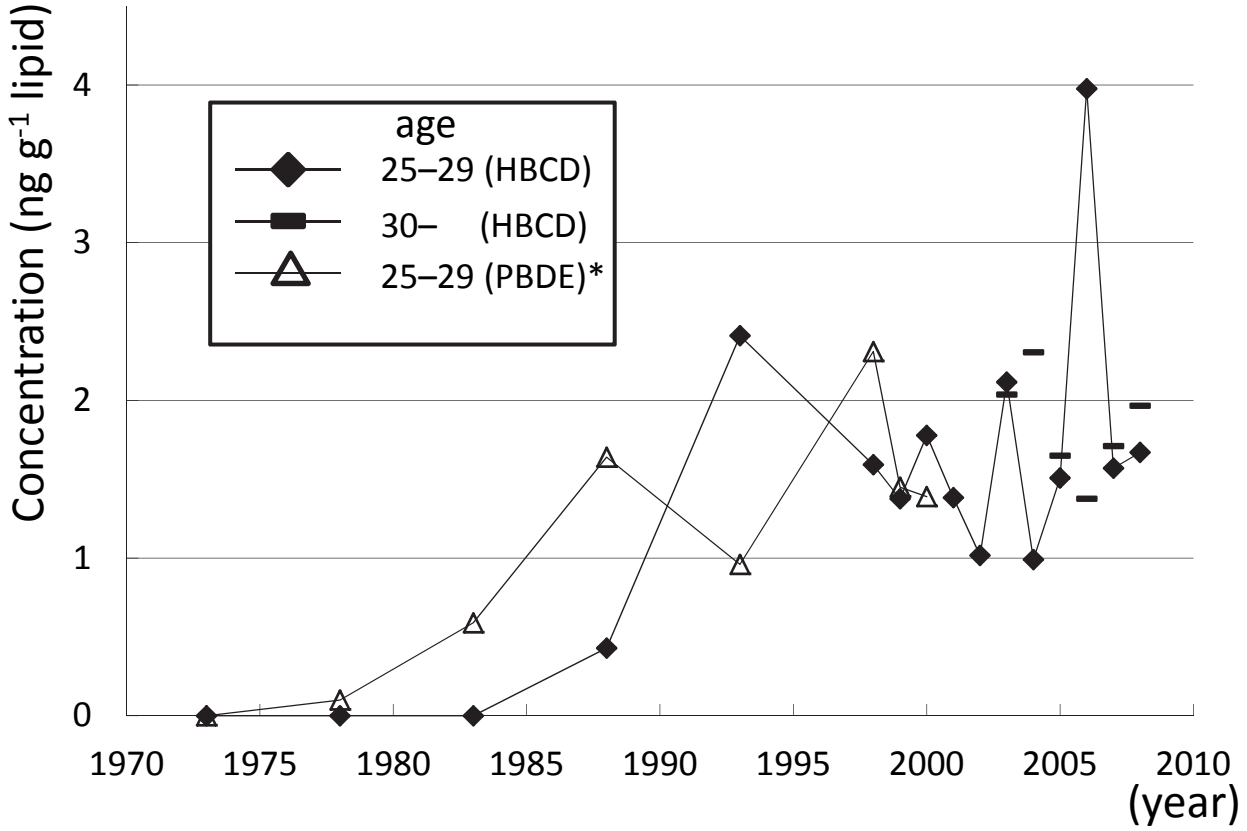


Fig.4 Time trend in the concentrations of ΣHBCD (α, β, andγ stereoisomers) and Σ PBDE in the breast milk of Japanese women.
*13ΣPBDEs; #28, #37, # 75, # 47, # 66, # 77, # 100, # 99, #85, # 154, # 153, # 138, and # 183 (Akutsu *et al*. 2003)

$$U_{milk} = \frac{C_{milk} \times B_{ing} \times IR_{milk} \times F_{milk}}{BW_{infant}}$$

(μg/kg/day)

B_{ing}: bioavailability (100/100=1)
IR_{milk}: intake rate (0.8 kg/day)
F_{milk}: fat (3.7%)
BW_{infant}: body weight (6 kg)

(WHO. 1998)

Fig.5 Mathematical formula in EU risk assessment report for estimation of exposure level

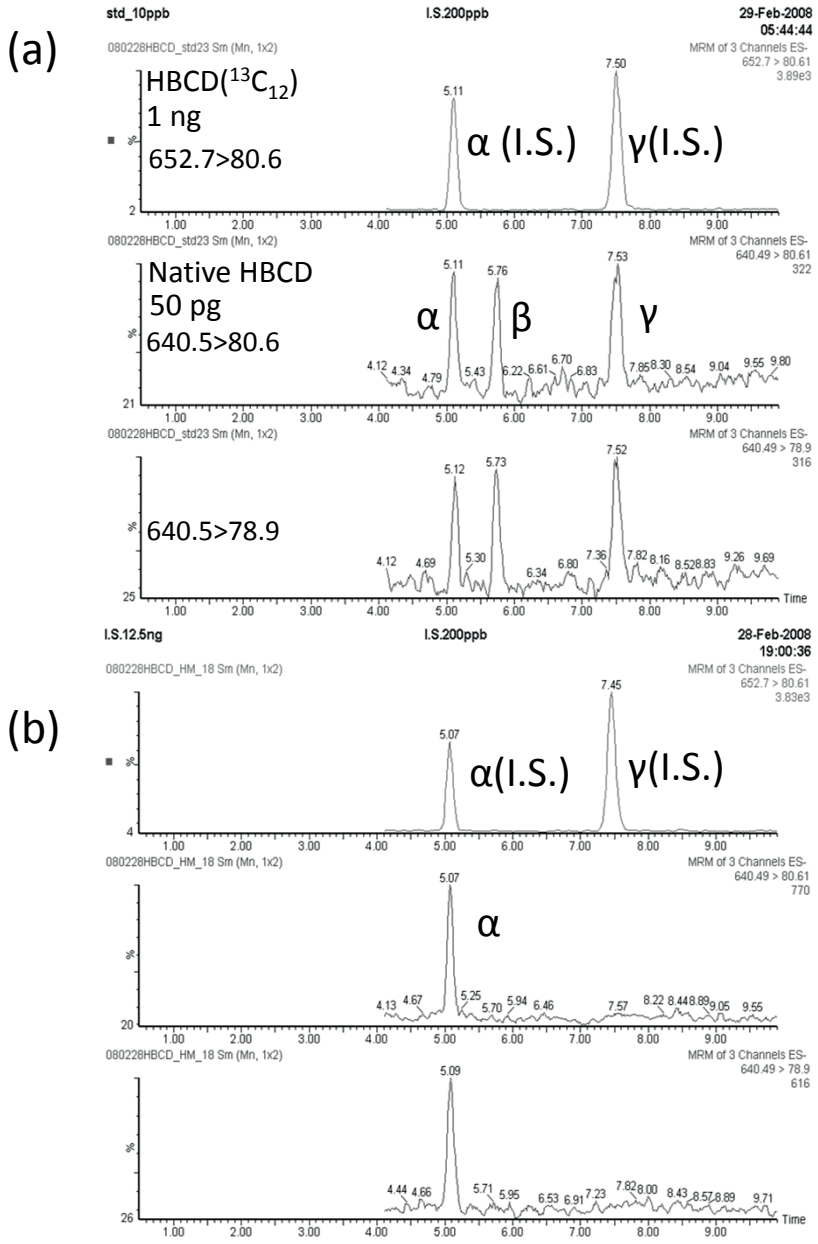
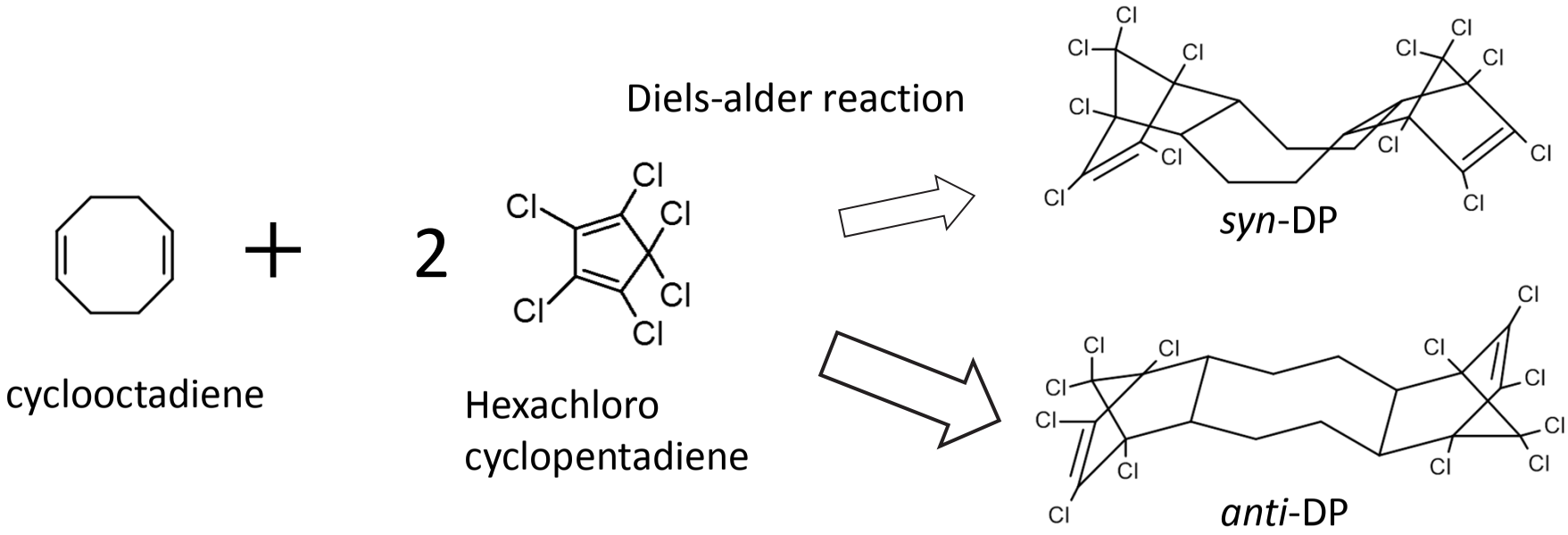
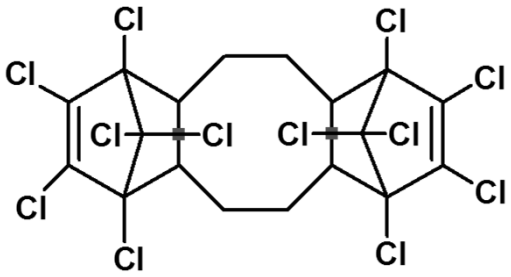


Fig.6 Representative chromatograms HBCD standard (a) and breast milk sample (b)





Physical properties of dechlorane plus.

Property	Value
Chemical formula	$C_{18}H_{12}Cl_{12}$
Molecular mass	653.7
Melting point	350 °C with decomposition
Vapor pressure (at 200 °C)	0.006 mm of Hg
Water solubility	44 ng L^{-1}–249 μg L^{-1}
Octane–water partition coefficient (Log Kow)	9.3

Data cited from US EPA, 2011; Oxychem, 2011.

Fig.7 Chemical structure and properties of Dechlorane Plus

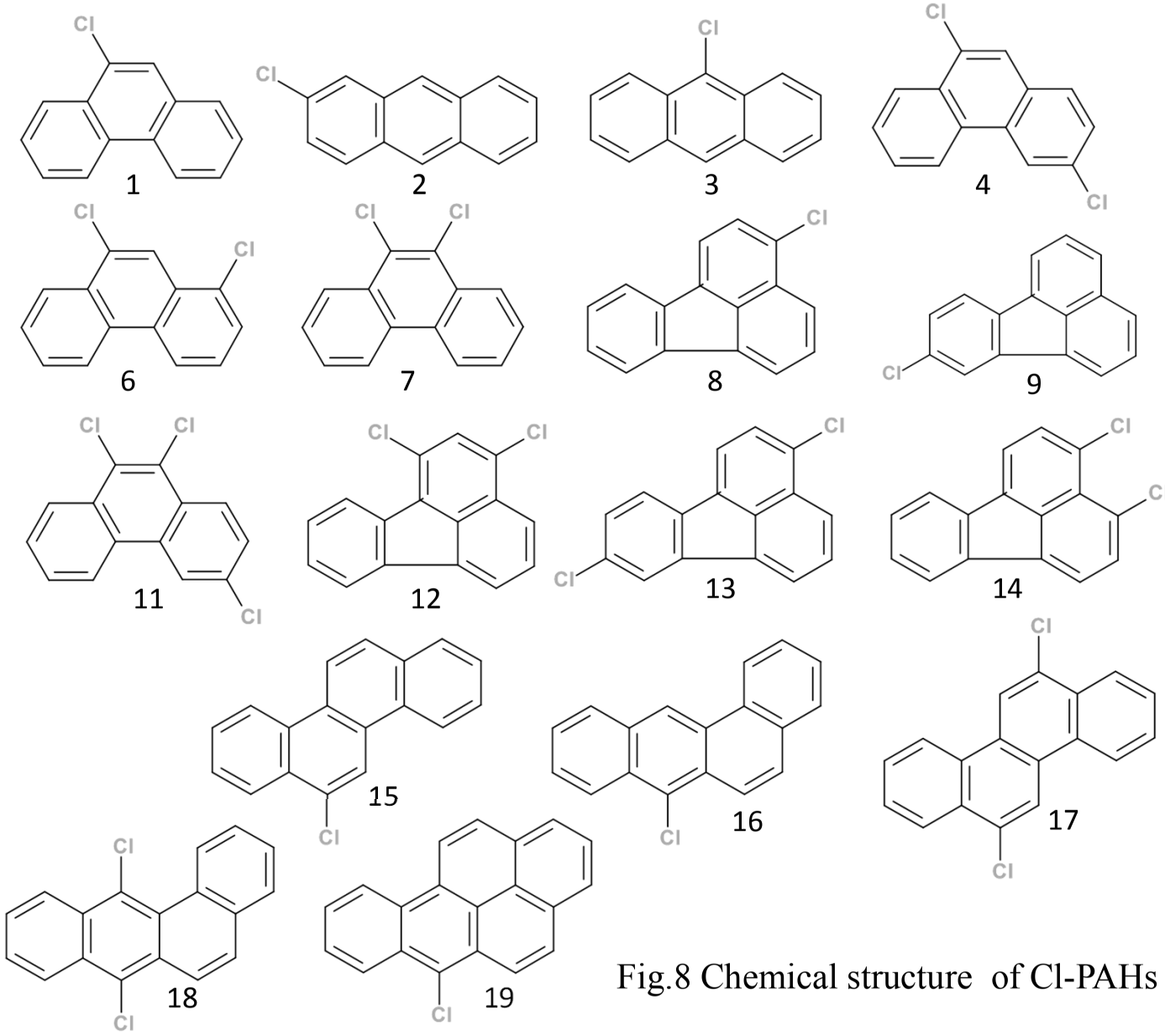


Fig.8 Chemical structure of Cl-PAHs

No.	Compound
1	9-chlorophenanthrene
2	2-chloroanthracene
3	9-chloroanthracene
4	3,9-dichlorophenanthrene
5	9,10-dichloroanthracene
6	1,9-dichlorophenanthrene
7	9,10-dichlorophenanthrene
8	3-chlorofluoranthene
9	8-chlorofluoranthene
10	1-chloropyrene
11	3,9,10-trichlorophenanthrene
12	1,3-dichlorofluoranthene
13	3,8-dichlorofluoranthene
14	3,4-dichlorofluoranthene
15	6-chlorochrysene
16	7-chlorobenz[a]anthracene
17	6,12-dichlorochrysene
18	7,12-dichlorobenz[a]anthracene
19	6-chlorobenzo[a]pyrene

2. Findings on human exposure of halogenated organic pollutants through food and atmosphere

2.1. Detection of Dechlorane Plus and brominated flame retardants in marketed fish in Japan

2.1.1. Abstract

Fish samples purchased from Japanese supermarkets were analyzed for Dechlorane Plus (DP) (*syn*-, *anti*-), polybrominated diphenyl ether (PBDE) and hexabromocyclododecane (HBCD) (α, γ). Twenty fish were analyzed using gas chromatography-mass spectrometry for DP and PBDE, and liquid chromatography-tandem mass spectrometry for HBCD. DP was detected in 18 samples and ΣDP concentrations were <0.2 to 14.2 pg g^{-1} wet wt. Among the DP isomers, *anti*-DP was the dominant residue observed in this study. PBDE was detected in all samples. Concentrations of ΣPBDE ranged from 2.2 to 878 pg g^{-1} wet wt. HBCD was detected in 18 samples, and ΣHBCD concentrations were <0.02 to 21.9 ng g^{-1} wet wt. In fish landed from the East China Sea and the Sea of Japan, we detected relatively high concentrations of DP, PBDE, and HBCD. These results indicate that the seawaters around East Asia are contaminated with flame retardants. This study shows for the first time that DP exists in fish marketed in Japan.

2.1.2. Introduction

In recent years, there has been increasing concern regarding worldwide pollution caused by halogenated flame retardants. PBDEs, HBCD, and DP have characteristics typical of bio-accumulative, persistent organic pollutants. In 2009, penta-BDE and octa-BDE were included in the Conference of the Parties of the Fourth Stockholm Convention on POPs (www.pops.int).

Dechlorane Plus is a highly chlorinated additive flame retardant, and has a cost advantage over comparable brominated flame retardants, such as PBDEs and HBCD. It has also been identified by the European Commission as a possible replacement for BDE-209 (Pakalin et al., 2007). DP has been manufactured for more than 40 years by OxyChem in Niagara Falls, NY and is used in electrical wire and cable coatings, computer connectors and plastic roofing materials (Tomy et al., 2007). The US EPA lists it as a high-production-volume (HPV) chemical, and toxicity studies were performed according to the HPV test plan (US EPA, 2004). However, the toxicity information in this test plans (US EPA, 2009; Brock et al., 2010) was limited in terms of environmental fate and chronic toxicity. In addition to the toxicity information from the manufacturer, there are a few studies into the effects of cytotoxicity, mRNA expression (Crump et al., 2011), blood-brain barrier permeability of *anti*-DP in fish (Zhang et al., 2011c), induction of hepatic oxidative damage, perturbations of metabolism, and signal transduction in a mouse model (Wu et al., 2012).

There are concerns that DP accumulates in the environment and in living systems because of its typical characteristics as a persistent organic pollutant. For example, it has a very high octanol-water partition coefficient (Log Pow = 9.3) (US EPA, 2004).

The first detection of DP from the environment near the manufacturing plant was

reported in 2006 (Hoh et al., 2006). Since this report, DP has been detected near the manufacturing area(Sverko et al., 2008; Gauthier and Letcher, 2009; Wang et al., 2013) and even in environmental matrices (air, water, and soil) (Ren et al., 2008; Qi et al., 2010; Sakiyama and Nakano, 2012) and biological matrices (fish, bird, human serum, human milk, and hair) (Ren et al., 2009; Kang et al., 2010; Zheng et al., 2010; Zhang et al., 2011a; Siddique et al., 2012) in a region remote from the manufacturing area. The detection of DP has especially increased in China in recent years. Possible contributing factors are the recently reported DP production plant, Jiangsu Anpon Electrochemical Company, in Huai'an City (Wang et al., 2010; Zhang et al., 2013) and electronic waste recycling areas (Wu et al., 2010; Wang et al., 2011; Zhang et al., 2011b) in China. The reports about highly DP polluted areas, such as manufacturing sites in the US and China, and electronic-waste recycling sites, are listed in Table 4 (Hoh et al., 2006; Qiu et al., 2007; Zhu et al., 2007; Wu et al., 2010; Wang et al., 2010; Zhang et al., 2013).

DP is synthesized from cyclooctadiene and hexachlorocyclopentadiene through the Diels–Alder reaction where the *syn*- and *anti*- isomers are produced (Fig. 7). The major isomer in commercial products is *anti*-DP (Hoh et al., 2006). The isomeric ratio of detected DP varies depending on the analysis samples.

Japan's geographical location makes it susceptible to air currents (Westerlies) or ocean

currents (Tsushima Current) from the Asian continent. At the 20th Symposium on Environmental Chemistry, held in Japan in 2011, Sakiyama and Nakano reported the presence of DP in Japanese soil and dust samples. However, no data are available on the level of human exposure to DP in Japan. The primary routes for non-occupational exposure to environmental pollutants are diet and inhalation. Meng et al. (2007) reported that the level of exposure to PBDE in China was generally similar for inhalation and fish consumption routes.

To determine the DP pollution level in Japan, the fish sold in Japanese supermarket were analyzed for preliminary investigation because POPs (Fig. 1) tend to accumulate more over time in fish than in other food types. We also determined the levels of other halogenated flame retardants, such as PBDE and HBCD, for a comprehensive assessment of flame retardant contamination.

2.1.3. Materials and methods

2.1.3.1. Sample preparation

The fish samples were purchased from supermarkets in Osaka, Japan in June 2011 (Table 5). Body muscles were collected from the fish and chopped in a conventional food processor for 3 min to produce thoroughly mixed homogenates. The homogenized

samples were then frozen and stored until use.

2.1.3.2. Chemicals

The non-labelled DP (*syn*- and *anti*-), PBDE and HBCD (α and γ), $^{13}C_{12}$-labelled PBDE, $^{13}C_{12}$-labelled HBCD (α and γ) standards were purchased from Wellington Laboratories (Ontario, Canada). The $^{13}C_{10}$-labelled 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).

2.1.3.3. Extraction and clean-up

The 15 g samples were spiked with ^{13}C-labeled standard mixtures ($^{13}C_{10}$-labeled DP, (*syn*- and *anti*-), 0.25 ng each; $^{13}C_{12}$-labeled PBDE, 0.25–0.5 ng; $^{13}C_{12}$-labeled HBCD, (α and γ), 1.25 ng each). The samples were extracted using a mixture of n-hexane and diethyl ether (2:1, v/v), twice using a homogenizer followed by centrifugation. The organic layer was collected and washed with 2%NaCl. After dehydration with Na_2SO_4, the organic solvent was concentrated using a rotary evaporator until the organic solvent was completely removed, 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. This fraction was evaporated to dryness and redissolved in hexane. The solution was subjected to a cleanup procedure using a 44% sulfuric acid-impregnated silica gel column (1 g). Hexane (10 ml) was used as an eluent. After adding 50 μl of nonane, the eluate was evaporated until the hexane was completely removed. Finally, 1 μl of sample solution was used for the analyses of DP and PBDE by gas chromatography-mass spectrometry (GC/MS). For HBCD analyses, nonane was removed under an N_2 stream and 50 μl of methanol added, then 5 μl of the sample solution was used for the liquid chromatography-tandem mass spectrometry (LC/MS/MS) analysis.

2.1.3.4. GC/MS conditions

GC/MS analyses were performed using a gas chromatograph (6890 series, Agilent Technologies, CA, USA) coupled to a high-resolution mass spectrometer (JMS-700D, 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°C (held for 2 min) to 330°C (held for 5 min) at 10°C min^{-1}; carrier gas, helium; column head pressure, 10 psi; injection temperature, 250°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, 600 μA; ion source temperature, 270°C; resolution, 10,000 (10% valley definition). The monitoring ions for each target compound are shown in Table 6. The DP and PBDE were quantified by the isotope dilution method using the corresponding ^{13}C-labeled congeners.

2.1.3.5. LC/MS/MS conditions

LC/MS/MS analyses were performed on a liquid chromatograph (ACQUITY UPLC system, Waters) coupled to a tandem quadrupole mass spectrometer (Quattro Premier XE, Waters). Chromatographic separation of the HBCD isomers was performed on a C18 column (ACQUITY UPLC BEH C18, 100 mm, 2.1 mm i.d., 1.7 μm, Waters). The column temperature was maintained at 40°C. Isocratic elution was then performed using 10 mM ammonium acetate in a mixture of acetonitrile, water, and methanol (65:23:12) at a flow rate of 0.2 ml min^{-1}.

The MS conditions were as follows: ionization mode, electrospray ionization mode

(negative); capillary voltage, 2800 V; extractor voltage, –2 V; cone voltage, –16 V; collision energy, 9 eV. The selected reaction monitoring mode was used for quantitation; *m/z* 640.5 to 80.6 transition for non-labeled HBCD and *m/z* 652.7 to 80.6 for $^{13}C_{12}$-labeled HBCD. The *m/z* 640.5 to 78.9 transition was verified further. The HBCD isomers were quantified by the isotope dilution method using the corresponding ^{13}C-labelled congeners.

2.1.3.6. Quality assurance and quality control

Spiked skipjack-tuna sample (n = 5) recoveries of DP, PBDE and HBCD are shown in Table 7. The spiking amounts were as follows: DP (*syn*- and *anti*-), 0.3 ng each; PBDE, 0.2–0.4 ng; HBCD (α and γ), 1 ng each. A procedural blank was run for each batch of fish samples. The calculated detection limits (*S*/*N* = 3) of DP, PBDE and HBCD were 0.2 pg g^{-1} wet wt, 1.0–6.0 pg g^{-1} wet wt, and 0.02 ng g^{-1} wet wt respectively. Quality control standards were measured after every 10 samples to check for instrumental drift. None of the analytes was detected in the procedural blank samples.

2.1.4. Results and discussion

The concentrations of DP, PBDE, and HBCD in the fish samples are summarized in Table 8. DP was detected in 18 samples and ΣDP concentrations were < 0.2 to 14.2 pg

g^{-1} wet wt. Representative chromatograms are shown in Fig. 9. PBDE was detected in all samples with concentrations between 2.2 and 878 pg g^{-1} wet wt for ΣPBDE. Among the congeners, BDE-47 is known as a common indicator of PBDE contamination in marine fish (Akutsu et al., 2001) and it was also the most dominant congener, detected in all samples in the study. HBCD was detected in 18 samples with ΣHBCD concentrations of < 0.02 to 21.9 ng g^{-1} wet wt. The concentrations of ΣPBDE were highest in the mackerel landed at Fukuoka (sample No. 8). ΣHBCD and ΣDP were also relatively high in this sample. Fukuoka prefecture faces onto the East China Sea, which is southwest of Japan and is bordered by Japan, Korea, and China. ΣDP was highest in the Japanese seaperch landed at Hyogo (sample No. 13) and ΣHBCD was highest in the Japanese Spanish mackerel landed at Kyoto (sample No. 3). Both prefectures face the Sea of Japan, which is northwest of Japan and bordered by Japan, Korea, and Russia. These results indicate that the seawaters around Japan, Korea, Russia and China are contaminated by flame retardants. Among the fish species, DP, PBDE, and HBCD concentrations were very low in skipjack tuna, sea bream, and Pacific cod, independent of the landing location.

When we assess the correlation of concentrations between ΣDP, ΣPBDE, and ΣHBCD, DP does not correlate with PBDE or HBCD ($r = 0.20$, $\rho = 0.41$ and $r = 0.30$, $\rho = 0.20$

respectively), but there is a weak correlation between PBDE and HBCD ($r = 0.65$, $\rho < 0.01$) (Fig. 10). This correlation may be due to their similar intended end-usage and contamination sources (industrial effluent, waste disposal site). Both PBDE and HBCD are brominated flame retardants and used as a protective additive to polystyrene foam (upholstery and building materials), textiles (furniture, mattresses, and curtains), and electrical equipment (de Wit, 2002).

As a result of the fast development of the electrical industry in recent years, electronic waste (e-waste) generated throughout the world is now being transported to developing countries for recycling. Bi et al., (2007) reported that computer e-waste is exported to Asia, primarily to China. Several flame retardants have been detected in water birds from an e-waste recycling region (Zhang et al., 2011a). These facts may contribute to the high environmental levels of flame retardants found in seawater around eastern Asia.

The fractional abundance of the *anti*-isomer (f_{anti}) was calculated by dividing the concentration of *anti*-DP by the sum of the concentrations of *syn*- and *anti*-DP. The mean f_{anti} value in this study was 0.62 ± 0.05 (mean ± one standard deviation) and is similar to that of fish from rivers in Korea (0.69) (Kang et al., 2010). The f_{anti} values of commercial DP products were 0.75–0.80 for the Great Lakes area (Hoh et al., 2006) and 0.70 in China (Wu et al., 2010). Some reports indicate that *syn*-DP is more

bio-accumulative than *anti*-DP, and there is a tendency towards higher trophic levels, hence the higher *syn*-DP ratio in aquatic biota (Wu et al., 2010; Xian et al., 2011; Wang et al., 2015). In this study, the f_{anti} value (0.56-0.72) was rarely different from that of the commercial product.

For HBCD, α-HBCD was the dominant isomer in almost all samples. In the technical mixture, γ-HBCD is the most dominant isomer (> 70%) followed by α-HBCD (Tomy et al., 2004). In marine fish/mammals, α-HBCD is reported to be the dominant residue among the HBCD isomers (Covaci et al., 2006) because of its relatively longer environmental and biological half-life compared to γ-HBCD. DP and HBCD isomer profiles from the results in this study indicate that, with respect to biological accumulation, HBCD has more pronounced isomer selectivity than DP.

The daily intakes of ΣDP, ΣPBDE, and ΣHBCD from fish by an average Japanese adult were tentatively calculated to be 2.4 pg kg^{-1} body weight day^{-1}, 115 pg kg^{-1} body weight day^{-1}, and 2.2 ng kg^{-1} body weight day^{-1}, respectively. Comparing these values to the reported estimated daily intake from fish, PBDE was comparable to the reported value in the UK (400 pg kg^{-1} body weight day^{-1}) (UK Food Standards Agency, 2006), and the Netherlands (220 pg kg^{-1} body weight day^{-1}) (Bakker et al., 2008) and HBCD was comparatively higher than the reported value in the UK (0.3 ng kg^{-1} body weight day^{-1})

(UK Food Standards Agency, 2006), and the Netherlands (0.12 ng kg^{-1} body weight day^{-1}) (van Leeuwen and de Boer, 2008).

2.1.5. Interim summary

In conclusion, this study has shown for the first time that DP is being detected in fish in Japan, despite there being no DP manufacturing facility in Japan. Concentrations of ΣDP were approximately one hundredth of those of ΣPBDE and one thousandth of those of ΣHBCD. Although these concentrations in fish samples would not be expected to cause adverse effects in humans, further monitoring and research of dietary products focused on more food variety are needed amid mounting concerns about contamination by DP. This is of particular importance because DP has the typical characteristics of a persistent organic pollutant.

Table 5
Details of fish from supermarket in Osaka, Japan

No.	Fish species	Landing prefecture, Country
1	Japanese spanish mackerel	Hyogo, Japan
2	Japanese spanish mackerel	Toyama, Japan
3	Japanese spanish mackerel	Kyoto, Japan
4	Skipjack tuna	Kochi, Japan
5	Skipjack tuna	Miyagi, Japan
6	Sea bream	Fukuoka, Japan
7	Sea bream	Fukuoka, Japan
8	Mackerel	Fukuoka, Japan
9	Mackerel	Chiba, Japan
10	Pacific bluefin tuna	Nagasaki, Japan
11	Tongue-sole	Hyogo, Japan
12	Chicken grunt	Wakayama, Japan
13	Japanese seaperch	Hyogo, Japan
14	Olive flounder	Aomori, Japan
15	Japanese sardine	Chiba, Japan
16	Japanese jack mackerel	Hyogo, Japan
17	Flathead flounder	Ishikawa , Japan
18	pacific cod	Hokkaido , Japan
19	Greater amberjack	Unknown, Japan
20	Greenland halibut	Unknown, Denmark

Table 6
Monitor ions for DP and PBDE used in this study

Compounds	Monitor ions (*m/z*)		Surrogate I.S.[a]
	Quantification	Qualification	
syn-DP	271.8102	273.8072	$^{13}C_{10}$- *syn*-DP
anti-DP	271.8102	273.8072	$^{13}C_{10}$- *anti*-DP
$^{13}C_{10}$- *syn*-DP	276.8269	278.8240	-
$^{13}C_{10}$- *anti*-DP	276.8269	278.8240	-
BDE-28	405.8027	407.8006	$^{13}C_{12}$-BDE-28
BDE-49	485.7111	483.7132	$^{13}C_{12}$-BDE-47
BDE-47	485.7111	483.7132	$^{13}C_{12}$-BDE-47
BDE-66	485.7111	483.7132	$^{13}C_{12}$-BDE-47
BDE-100	563.6216	565.6196	$^{13}C_{12}$-BDE-99
BDE-99	563.6216	565.6196	$^{13}C_{12}$-BDE-99
BDE-126	563.6216	565.6196	$^{13}C_{12}$-BDE-99
BDE-154	643.5301	641.5321	$^{13}C_{12}$-BDE-153
BDE-153	643.5301	641.5321	$^{13}C_{12}$-BDE-153
BDE-184	721.4406	723.4386	$^{13}C_{12}$-BDE-183
BDE-183	721.4406	723.4386	$^{13}C_{12}$-BDE-183
BDE-197	641.5144	639.5165	$^{13}C_{12}$-BDE-197
BDE-196	641.5144	639.5165	$^{13}C_{12}$-BDE-197
BDE-207	719.4250	721.4229	$^{13}C_{12}$-BDE-207
BDE-206	719.4250	721.4229	$^{13}C_{12}$-BDE-207
BDE-209	799.3334	797.3355	$^{13}C_{12}$-BDE-209
$^{13}C_{12}$-BDE-28	417.8429	419.8409	-
$^{13}C_{12}$-BDE-47	497.7514	495.7534	-
$^{13}C_{12}$-BDE-99	575.6619	577.6598	-
$^{13}C_{12}$-BDE-154	655.5704	653.5724	-
$^{13}C_{12}$-BDE-153	655.5704	653.5724	-
$^{13}C_{12}$-BDE-183	733.4809	735.4788	-
$^{13}C_{12}$-BDE-197	653.5547	651.5567	-
$^{13}C_{12}$-BDE-207	731.4652	733.4632	-
$^{13}C_{12}$-BDE-209	811.3737	809.3757	-

[a] Surrogate internal standard used for quantification of each native compounds

Table 7 Results of recovery test for DP, PBDE, and HBCD

Compounds	Recovery(%)	R.S.D.(%)
syn-DP	106	1.0
anti-DP	101	0.9
BDE-28	103	2.3
BDE-49	106	9.6
BDE-47	105	3.0
BDE-66	102	1.7
BDE-100	101	8.0
BDE-99	94	2.6
BDE-126	100	10.4
BDE-154	110	3.6
BDE-153	101	3.7
α-HBCD	107	8.3
γ-HBCD	100	5.6

Table 8 Concentrations of DP, PBDE, and HBCD in fish

	DP (pg g^{-1} wet wt)				PBDE (pg g^{-1} wet wt)										HBCD (ng g^{-1} wet wt)		
No.	*syn*-	*anti*-	ΣDP	f_{anti}[a]	#28	#49	#47	#66	#100	#99	#126	#154	#153	ΣPBDE	α	γ	ΣHBCD
1	0.67	0.84	1.51	0.56	7.7	7.4	25.3	4.4	N.D.	3.2	N.D.	3.5	N.D.	52	0.31	0.27	0.58
2	1.27	1.87	3.14	0.59	6.0	21.0	39.9	2.3	11.1	7.1	2.5	24.6	N.D.	115	2.73	1.15	3.88
3	1.10	1.56	2.66	0.59	16.9	68.2	119	9.5	43.7	20.9	18.7	55.4	11.7	364	9.67	12.2	21.9
4	N.D.	0.37	0.37	-	N.D.	2.1	2.5	N.D.	N.D.	N.D.	N.D.	5.8	N.D.	10	0.02	N.D.	0.02
5	N.D.	0.28	0.28	-	N.D.	2.8	2.5	N.D.	N.D.	N.D.	N.D.	2.8	N.D.	8	0.04	N.D.	0.04
6	N.D.	0.62	0.62	-	N.D.	1.5	3.7	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	5	0.03	N.D.	0.03
7	N.D.	0.45	0.45	-	N.D.	N.D.	3.0	N.D.	N.D.	N.D.	N.D.	3.8	N.D.	7	N.D.	N.D.	-
8	0.92	1.68	2.60	0.65	11.7	112	265	20.8	91.1	89.1	15.9	204	68.7	878	6.08	3.29	9.37
9	0.33	0.83	1.16	0.72	1.7	9.2	16.1	N.D.	5.5	3.5	N.D.	12.4	N.D.	48	0.48	0.06	0.54
10	0.54	1.04	1.58	0.66	9.2	76.3	161	18.4	67.9	38.7	11.6	118	27.3	529	2.48	1.37	3.85
11	0.59	0.78	1.37	0.57	N.D.	N.D.	3.5	N.D.	N.D.	N.D.	N.D.	2.6	N.D.	6	N.D.	N.D.	-
12	N.D.	N.D.	-	-	N.D.	1.7	4.0	N.D.	N.D.	N.D.	N.D.	3.5	N.D.	9	0.03	N.D.	0.03
13	6.10	8.09	14.2	0.57	17.4	25.7	89.3	9.4	16.2	5.1	3.1	32.6	6.8	206	5.50	1.16	6.66
14	N.D.	0.70	0.70	-	17.9	109	291	8.8	62.6	21.4	15.8	84.0	19.8	631	8.68	0.91	9.59
15	1.62	2.47	4.09	0.61	4.3	17.2	31.6	2.4	7.3	6.4	9.6	15.9	N.D.	95	0.83	0.29	1.12
16	1.54	2.15	3.69	0.58	2.8	8.1	21.6	N.D.	8.3	N.D.	6.8	9.4	N.D.	57	0.58	0.13	0.71
17	0.25	0.54	0.79	0.68	1.0	11.0	28.0	N.D.	6.4	N.D.	3.3	13.9	N.D.	64	N.D.	0.07	0.07
18	N.D.	N.D.	-	-	N.D.	N.D.	2.2	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	2	0.03	N.D.	0.03
19	1.76	3.23	4.99	0.65	17.3	100	199	4.5	106	39.3	41.4	138	24.0	670	6.68	0.36	7.04
20	N.D.	0.44	0.44	-	9.4	19.4	144	4.4	19.6	15.8	N.D.	10.4	N.D.	223	0.09	0.05	0.14

Abbreviations: N.D., not detected (< 0.2 pg g^{-1} wet wt for *syn*- and *anti*-DP;< 1.0 pg g^{-1} wet wt for BDE-28, BDE-49, BDE-47, BDE-99; < 2.0 pg g^{-1} wet wt for BDE-66, BDE-126, BDE-154; < 4.0 pg g^{-1} wet wt for BDE-100; < 6.0 pg g^{-1} wet wt for BDE-153; < 0.02 ng g^{-1} wet wt for α-and γ-HBCD)

The concentrations of total PBDE, total HBCD and total DP were calculated by assuming the non-detected values as zero.

[a] f_{anti} value was calculated in cases of both *syn*- and *anti*-DP were detected.

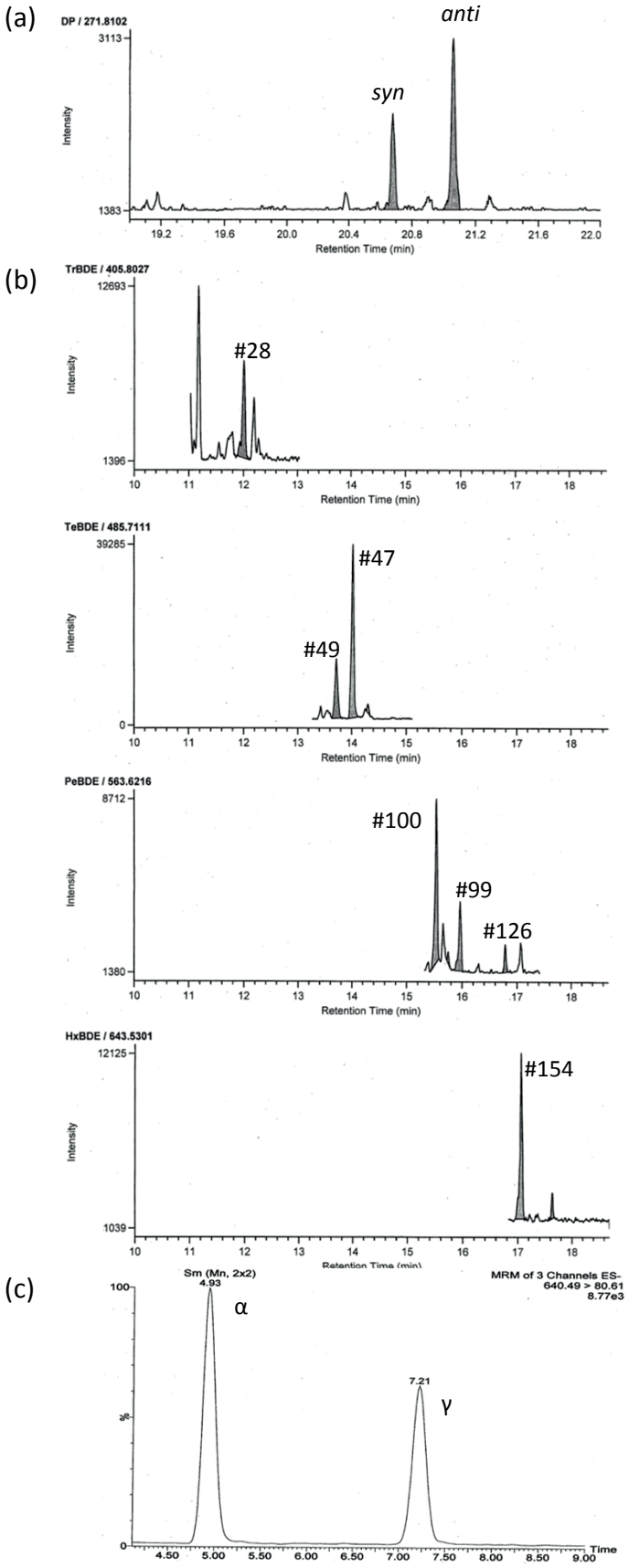
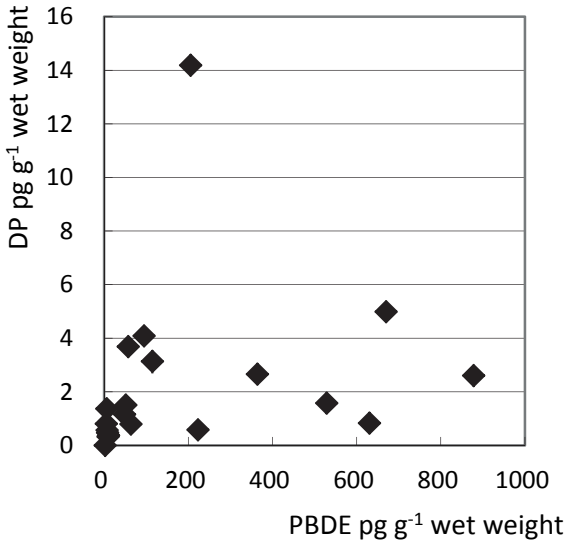
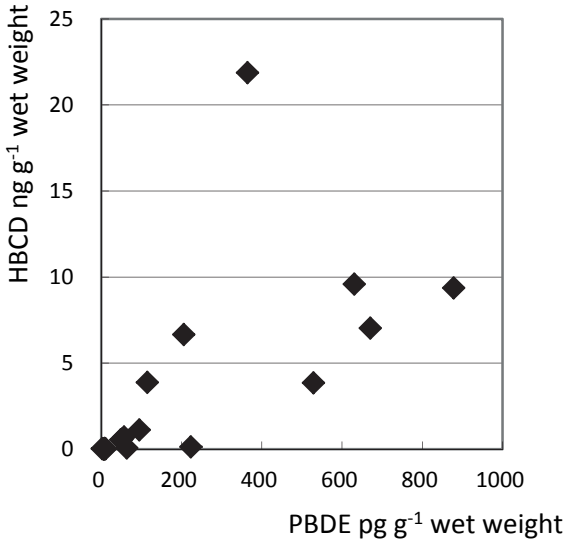


Fig.9 Chromatogram of Fish sample（Sample No.2）
(a) DP, (b) PBDE, and (c) HBCD





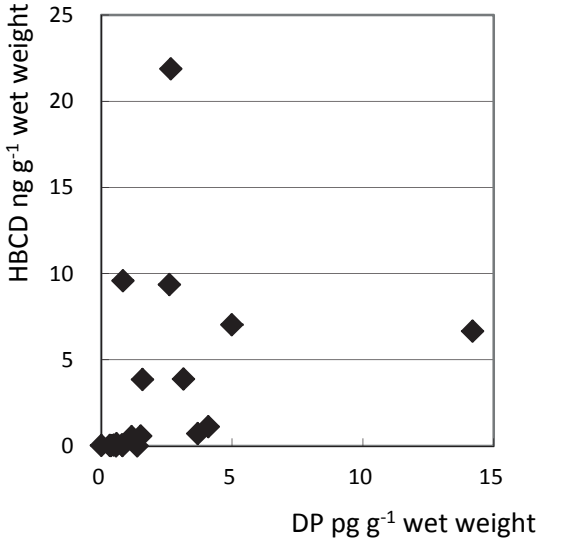


Fig.10 Correlation of concentrations among ΣDP, ΣPBDE and ΣHBCD

2.2. Exposure level evaluation for Dechlorane Plus in Osaka, Japan

2.2.1. Abstract

This study estimated daily exposure to Dechlorane Plus (DP) and polybrominated diphenyl ethers (PBDE) via inhalation and diet. Samples of atmospheric particles and food (obtained by the market basket method) from Osaka, Japan were analyzed for DP (*syn*-, *anti*-) and PBDE using gas chromatography-mass spectrometry. DP was detected in both atmospheric particles and food samples. Among the atmospheric particles, DP was detected in all samples. ΣDP concentration was 7.1–15.4 pg m^{-3} and *anti*-DP was the dominant residue among DP isomers. PBDE was also detected in all the atmospheric particles. ΣPBDE concentration was 9.9–23.3 pg m^{-3}. In the market basket study, DP was detected in Groups Ⅲ (sugar and confectionery), V (legumes and their products), X (fish, shellfish, and their products), and XI (meat and eggs) at concentrations of 3.3, 2.8, 1.9, and 1.5 pg g^{-1} wet wt, respectively. PBDE was detected in Groups Ⅲ, IV (oils and fats), V, X, XI, and XⅢ (seasonings and other processed foods) at concentrations of 153, 79.1, 74.6, 308, 94.8, and 186 pg g^{-1} wet wt, respectively. The daily intake of ΣDP (750 pg day^{-1}) via inhalation and diet was approximately one percent of that for ΣPBDE (62 ng day^{-1}).

2.2.2. Introduction

In a preliminary study, we detected DP in fish samples for the first time and determined its pollution status in Japan. On the basis of this result, we believe it is important to estimate the human DP exposure level in Japan. We therefore determined the inhalational and dietary intake amounts through the analysis of atmospheric particle samples and basket study samples collected in Osaka, Japan. In addition to DP, we also determined the exposure levels of PBDE.

2.2.3. Methods

2.2.3.1 Sample preparation

Atmospheric particle samples

Samples were collected in the vicinity of a research laboratory located in the eastern urban area of Osaka City, Japan. Osaka City is one of the biggest cities in Japan. Air samples were collected using a high-volume air sampler equipped with a quartz-fiber filter (QFF) at a flow rate of 0.5 m^3 min^{-1}. Sampling periods were November to December 2012, and February to March 2013. The QFF was replaced every week. After sampling, the filters were packaged with aluminum foil and stored in a freezer until analysis.

Market basket study samples

The market basket study samples were prepared in line with official food classification and consumption data from the National Nutrition Survey, conducted by the Ministry of Health, Labour and Welfare of Japan. A total of 123 food samples were purchased from supermarkets in Osaka in 2012. These food samples were cooked (boiled or broiled) in accordance with the guidelines provided by the Ministry of Health, Labour and Welfare of Japan and were then weighed according to daily consumption amounts. The weighed foods were mixed and homogenized to form 13 food-group composites numbered I to

XIII, as listed in Table 9. The homogenized samples were then frozen and stored until use.

2.2.3.2. Extraction and cleanup

Atmospheric particle samples

The filter samples were extracted ultrasonically for 30 min with 50 mL of dichloromethane containing ^{13}C-labeled standard mixtures ($^{13}C_{10}$-labeled DP, (*syn*- and *anti*-), 0.25 ng each; $^{13}C_{12}$-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 via gas chromatography-mass spectrometry (GC/MS).

Market basket study

The market basket study samples were treated using the same method as described previously for the fish sample analyses (section *2.1.3.*). Ten grams of each sample were used for extraction.

2.2.3.3. Quality assurance and quality control of filter samples

Analytical recoveries of the DP and PBDE in the filter samples (n = 5) ranged from

83% (BDE-126) to 108% (*syn*-DP) and relative standard deviations ranged from 3% to 24% (BDE-126) (spiking amount: DP (*syn*- and *anti*-), 0.25 ng each; PBDE, 0.2–1 ng each). The calculated detection limits of DP and PBDE were 0.01 pg m^{-3} and 0.02–0.12 pg m^{-3}, respectively ($S/N = 3$).

2.2.4. Results and discussion

2.2.4.1. Atmospheric particle samples

The concentrations of DP and PBDE in the filter samples are summarized in Table 10. 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}) (Ren et al., 2008) and the Great Lakes area near a DP manufacturing point (34 pg m^{-3}) (Hoh et al., 2006); and is higher than concentrations recorded at rural sites in China (3.5 pg m^{-3}) (Ren et al., 2008) and in the Shanghai area (5.48 pg m^{-3}) (Yu et al., 2011). Recently, very high atmospheric concentrations of DP were reported at an e-waste site (13.1−1,794 pg m^{-3}) (Chen et al., 2011) and a location near a DP production facility in China (7,737−26,734 pg m^{-3}) (Wang et al., 2010). Hoh et al. (2006) reported that atmospheric DP consisted almost entirely of particle phase, and that < 1% of total DP was present in gas-phase samples. We therefore consider that

the concentrations of ΣDP found in this study can represent DP levels in ambient air around Osaka, Japan. There is no DP manufacturing facility located in Japan, therefore potential sources of considerable atmospheric DP are likely to be via atmospheric transport across the sea from the Asian continent and release from DP-containing products used in Japan, such as electrical wire and cable coatings, computer connectors, and plastic roofing materials.

The fractional abundance of the *anti*-isomer (f_{anti}) was 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 ± one standard deviation) and was similar to both commercial DP products and atmospheric DP in China (0.63−0.68) (Ren et al., 2008; Wang et al., 2010)as well as urban Korea (0.82−0.88)(Baek et al., 2013). It is reported that the stereo-selective depletion of the *anti*-isomer was caused by UV radiation from sunlight during long-range atmospheric transport(LRAT)(Möller et al., 2010). We believe that the atmospheric DP in Osaka was little affected by long-range atmospheric transportation from remote DP sources, but further study is needed for LART estimation because in this study, we only analyzed the samples for Osaka, Japan.

PBDE was detected in all filter samples, at concentrations between 9.9 and 23.3 pg m^{-3} for ΣPBDE. These values were similar to the range 1.5–49 pg m^{-3} reported for

particulate samples investigated in Japan (Hayakawa et al., 2004). Among the PBDE congeners inthis study, BDE-209 was the most abundant in the atmosphere. One reason for the high concentrations of BDE-209 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 in both gas and particle phases. Strandberg et al. (2001) reported that the percentages present in the gas phase were about 80% for BDE-47, about 55–65% for BDE-100 and BDE-99, and about 30% for BDE-154 and BDE-153. 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 congeners (Hayakawa et al., 2004; Yang et al., 2012). We considered BDE-209 as a reasonably representative indicator of atmospheric PBDE contamination in this study.

2.2.4.2. Market basket study

The concentrations of DP and PBDE in the food samples are summarized in Table 11. 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 (sugar and confectionery), V

(legumes and their products), X (fish, shellfish, and their products), and XI (meat and eggs); and PBDE alone was detected in IV (oils and fats) and XIII (seasonings and other processed foods). Groups IV and XIII contained oil products of animal and plant origin. Total DP concentration was highest in the sugar and confectionary group. This may be caused by either the usage of DP contaminated raw materials, such as wheat and sugar, or contamination through the confectionary -making process in the plant. Wang et al reported that wheat was highly contaminated with DP in China (Wang et al., 2013). Total PBDE concentration was highest in the fish group (Group X) and BDE-47 was highest among the congeners in this group. BDE-47 is known as a common indicator of PBDE contamination in marine fish. With the exception of the fish group, BDE-209 was the most abundant. A recent dietary study also indicated that BDE-209 accounts for a large proportion of PBDE in food groups except for fish(UK Food Standards Agency, 2006; Chen et al., 2012).

The mean f_{anti} value was 0.62 ± 0.09 (mean ± one standard deviation), which is smaller than that of the atmospheric samples in this study. Some reports indicate that *syn*-DP is more bio-accumulative than *anti*-DP (Wu et al., 2010; Xian et al., 2011; She et al., 2013), and tends towards higher trophic levels, thereby explaining the higher *syn*-DP ratio in Group X (fish, shellfish, and their products).

2.2.4.3. DP and PBDE intake estimation

Assuming that DP showed the same level in outdoor air and indoor air, and that the mean inhalation rate for an adult is 15.7 m^3 day^{-1} (US EPA, 2011), we estimated the mean daily intakes of ΣDP and ΣPBDE for the sampling season as 174 pg day^{-1} and 238 pg day^{-1} , respectively. The dietary intakes of ΣDP (576 pg day^{-1}) and ΣPBDE (61.8 ng day^{-1}) by an average Japanese adult were estimated by multiplying the daily intake of a food group (Table 9) by the DP and PBDE concentration for that group (Table 11). 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. Dietary exposure levels of DP and PBDE estimated in this study were compared to recently reported values. Our DP estimate was significantly lower in comparison with Korea (11.2 ng day^{-1}) (Kim et al., 2014) while our PBDE estimate was similar to data for Korea (72.30 ng day^{-1})(Na et al., 2013) and Taiwan (67.95 ng day^{-1}) (Chen et al., 2012).

2.2.5. Interim summary

In conclusion, this is the first study to detect atmospheric DP in Japan, and has estimated daily exposure levels via inhalation and diet. These findings indicate that

Japanese residents are exposed to DP in everyday life. The atmospheric exposure to DP was around three-quarters that of PBDE; whereas, the dietary intake of DP was approximately one percent of that for PBDE. This result indicates that food contamination by DP is in an early phase in Japan. We have conclusively proven that DP persists and remains in the Japanese environment, and it is beginning to make the transition into the biota and food components.

Despite there being no DP manufacturing facility in Japan, the atmospheric concentration in Osaka, Japan is comparable to that reported for urban areas in China. In addition, the inhalational exposure ratio of DP is much higher than that of PBDE in Japan. When trying to find out whether the atmospheric DP contamination source in Japan is domestic or cross-border pollution by LRAT, it is necessary to analyze the atmospheric samples from multi-sampling points in a wide area because Japan's geographical location makes it susceptible to air currents (Westerlies) from the Asian continent.

Table 9 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
VII I	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

Table 10 Concentrations of DP and PBDE in atmospheric particles

	DP (pg m^{-3})				PBDE (pg m^{-3})															
filter[a]	*syn*-	*anti*-	ΣDP	f_{anti}	#28	#49	#47	#66	#100	#99	#126	#154	#153	#184	#183	#197	#196	#207	#206	#209	ΣPBDE
1	5.5	9.9	15.4	0.64	-	-	0.15	-	-	0.18	-	0.05	0.07	-	0.13	0.12	0.13	0.58	0.45	9.82	11.7
2	2.6	7.8	10.4	0.75	-	-	0.10	-	-	0.12	-	-	0.07	-	0.10	0.11	0.12	0.62	0.91	21.1	23.3
3	3.4	11	14.4	0.76	-	-	0.13	-	-	0.12	-	-	-	-	-	0.10	0.08	0.57	0.76	8.13	9.9
4	1.8	6.4	8.2	0.78	-	-	0.07	-	-	0.08	-	-	-	-	-	0.08	0.09	0.43	0.55	12.2	13.5
5	3.2	11	14.2	0.77	-	-	0.13	-	-	0.16	-	0.04	0.08	-	0.09	0.10	0.11	0.54	0.46	13.8	15.5
6	1.9	5.2	7.1	0.74	-	-	0.11	-	-	0.16	-	0.07	0.08	-	0.10	0.08	0.10	0.60	0.88	17.2	19.4
7	2.1	6.0	8.1	0.75	-	-	0.10	-	-	0.11	-	0.04	0.05	-	0.10	0.08	0.08	0.44	0.62	11.3	12.9

Non-detected compounds are indicated as (-) (detection thresholds < 0.01 pg m^{-3} for *syn*- and *anti*-DP; < 0.02 pg m^{-3} for BDE-28 ; < 0.04 pg m^{-3} for BDE-47, BDE-100, BDE-99, BDE-126, BDE154, BDE-153, BDE-184, BDE-197 and BDE-196; < 0.08 pg m^{-3} for BDE-49, BDE-66, BDE-183, BDE-207 and BDE-206; < 0.12 pg m^{-3} for BDE-209)
The concentrations of total DP and total PBDE were calculated by assuming the non-detected values as zero.
[a] sampling periods (1: *Nov. 21–28, 2012*; 2: *Nov. 29–Dec. 6, 2012*; 3: *Feb. 13–19, 2013*; 4: *Feb. 20–26, 2013*; 5: *Feb. 28–Mar. 7, 2013*; 6: *Mar. 7–14, 2013*; 7: *Mar. 14–21, 2013*)

Table 11 Concentrations of DP and PBDE in market basket study samples

Group No.	Composition	DP (pg g^{-1} wet wt)				PBDE (pg g^{-1} wet wt)																
		syn-	*anti*-	ΣDP	f_{anti}[a]	#28	#49	#47	#66	#100	#99	#126	#154	#153	#184	#183	#197	#196	#207	#206	#209	ΣPBDE
I	Rice and rice products	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II	Grains, seeds, and tubers	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
III	Sugar and confectionary	1.0	2.3	3.3	0.69	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	153	153
IV	Oils and fats	-	-	-	-	-	-	-	-	-	3.15	-	-	-	-	-	-	-	-	-	75.9	79.1
V	Legumes and their products	0.9	1.9	2.8	0.67	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	74.6	74.6
VI	Fruits	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
VII	Brightly colored vegetables	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
VIII	Other vegetables, mushrooms, and seaweeds	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
IX	Beverages	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
X	Fish, shellfish, and their products	1.0	0.9	1.9	0.49	6.88	56.10	88.15	-	31.64	24.46	9.43	47.05	12.16	-	-	-	-	-	-	31.8	308
XI	Meat and eggs	0.6	0.9	1.5	0.62	-	-	3.87	-	-	3.81	-	-	-	-	-	-	-	-	-	87.2	94.8
XII	Milk and dairy products	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
XIII	Seasonings and other processed foods	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	186	186

Non-detected compounds are indicated as (-) (detection thresholds < 0.4 pg g^{-1} wet wt for *syn*- and *anti*-DP; < 1.0 pg g^{-1} wet wt for BDE-28; < 2.0 pg g^{-1} wet wt for BDE-47, BDE-100, BDE-99, BDE-126, BDE154, BDE-153, BDE-184, BDE-197 and BDE-196; < 4.0 pg g^{-1} wet wt for BDE-49, BDE-66, BDE-183, BDE-207 and BDE-206; < 6.0 pg g^{-1} wet wt for BDE-209)
The concentrations of total DP and total PBDE were calculated by assuming the non-detected values as zero.
[a] f_{anti} value was calculated in cases of both *syn*- and *anti*-DP were detected.

2.3. Atmospheric level of Dechlorane Plus in East-Asian countries and particle size distribution

2.3.1. Abstract

Atmospheric particles were collected in both summer and winter in several cities in Japan (Sapporo, Sagamihara, Kanazawa, and Kitakyushu), Korea (Busan), and China (Beijing) using a high-volume air sampler equipped with a quartz-fiber filter. The samples were analyzed for Dechlorane Plus (DP) using gas chromatography high-resolution mass spectrometry. The samples from Kanazawa and Beijing were also analyzed for decabromodiphenyl ether (BDE-209). DP was detected in all samples. The mean ΣDP concentration was highest (6.7 pg m^{-3}) and lowest (0.87 pg m^{-3}) in the winter samples from Sagamihara and Busan, respectively. The study found that the seasonal variation of DP concentrations varied by sampling site. BDE-209 was detected in all the analyzed samples except for one of the Kanazawa winter samples. BDE-209 concentration was considerably higher in Beijing than in Kanazawa. Significant correlations were found between the concentrations of ΣDP and BDE-209 in the winter samples from Kanazawa and both summer and winter samples from Beijing. This similarity in the atmospheric behavior of DP and BDE-209, especially in winter, is assumed to reflect a common end usage and release mechanism. In the DP particle-size fraction distribution study, the highest DP concentration (~43% of ΣDP) was found in the finest particle group (<1.1 μm). For the f_{anti} value, there was a tendency for the value to decrease with the decreasing particle size.

2.3.2. Introduction

The purpose of this study was to compare the seasonal atmospheric levels of DP among Northeast Asian countries during the same season. It is possible to compare the atmospheric DP concentration level in different cities, and countries and discover the DP contamination source and its atmospheric behavior using the multipoint samples collected during the same periods. There is currently little information on DP

size-specific particle distribution, which is needed to better understand itsatmospheric fate. In this study, therefore, we used a high volume air sampler equipped with a five-stage porous jet nozzle for investigation of DP particle-size distribution behavior.

2.3.3. Methods

2.3.3.1. Sample preparation

Total suspended particle (TSP) samples were collected from six cities in Japan, Korea, and China. Detailed information about the sampling sites and periods is presented in Table 12. Sapporo is located in the northern part of Japan, and is the country's fourth largest city. Sagamihara borders Tokyo, Kanazawa is located centrally on the Sea of Japan side of the archipelago, and Kitakyushu is in the south of Japan. Busan is the second largest city in Korea and is located in the southern part of the country, while Beijing is the largest city in China and is located in the northeast of the country. Samples were collected using a high-volume air sampler equipped with a quartz-fiber filter at a flow rate of 1.0 m^3 min^{-1}. The quartz-fiber filter was replaced daily. After sampling, the filters were packaged in aluminum foil and stored in a freezer until analysis. We analyzed BDE-209 in addition to DP for the samples from Kanazawa and Beijing.

For the investigation of DP particle-size distribution behavior, size-classified particle matter was collected from 11 April 2014 to 14 April 2014 and 14 April 2014 to 21 April 2014 using a high volume air sampler equipped with a five-stage (> 7.0 μm; 7.0–3.3; 3.3–2.0; 2.0–1.1; < 1.1 μm porous jet nozzle (AH-600, Sibata scientific technology, Japan) at a flow rate of 0.566 m^3 min^{-1}.

2.3.3.2. Extraction and cleanup

The filter samples were treated using the same method described for the previous filter sample analyses (2.2.3.).

2.3.4. Results and discussion

The mean DP and BDE-209 particulate concentrations are shown in Table 13. DP was detected in all analyzed samples. The mean values of total DP isomers (ΣDP) were determined to be in the following order: Sapporo > Sagamihara >Kitakyushu > Kanazawa > Beijing > Busan. The samples from Sagamihara showed seasonal variation, while those from Sapporo showed relatively high concentrations in both summer and winter. In Sagamihara, the mean DP concentration in summer is more than quadruple the winter concentration. The ΣDP concentrations of samples collected in Japan, during this study were higher than those in Korea and China. Yang et al. (2013) reported the

correlation of annual average concentrations of atmospheric PBDE at 11 urban sites in China with high urban populations using a high-volume air sampler. In our previous study, in which we analyzed air particulate samples collected in Osaka, Japan, the ΣDP concentration was 11 pg m^{-3}. The population of Osaka City is 2.6 million, and when the ΣDP concentrations in five Japanese cities (including the data from Osaka) were plotted against the population, a good correlation (Fig. 11, $R^2 = 0.87$, $\rho < 0.01$) was obtained. This result indicates that in Japan the main DP contamination sources are closely linked with urban centers having dense populations. In China, high atmospheric DP concentrations were reported from DP contamination source areas, such as an e-waste site (13.1–1,794 pg m^{-3}) (Chen et al., 2011) and a location near a DP production facility in China (7,737–26,734 pg m^{-3}) (Wang et al., 2010). The mean DP concentration in the Beijing samples (1.98 pg m^{-3}) in this study was higher than that in the study using the high-volume air sampler in Harbin, China (0.4 pg m^{-3}) (Ma et al., 2011), and lower than that in Dalian, China (3 pg m^{-3}) (Yang et al., 2012), and Shanghai, China (2.32–5.48 pg m^{-3}) (Yu et al., 2011). In China, the DP concentrations seemed to decrease with increasing distance from the DP manufacturing plant in Huai'an City.

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 0.63–0.73, and f_{anti} values for each sampling site are summarized in Fig. 12 as box plots, which were produced using R statistical software program version 3.0.3. It seems that the f_{anti} values were slightly higher in Japan than in Busan. It has been reported that stereo-selective depletion of the *anti*-isomer can be caused by UV radiation from sunlight during long-range atmospheric transport (Möller et al., 2010; Salamova and Hites, 2011). The mean f_{anti} values in Japan ranged from 0.69 to 0.73 and were similar to those of DP commercial products. Therefore, we assumed that the atmospheric DP in Japan is minimally affected by long-range atmospheric transportation (LRAT) from remote DP sources. Alternatively, the DP in the Busan sample (f_{anti} = 0.63) possibly was affected by LRAT. BDE-209 was also analyzed for the samples from Kanazawa and Beijing, and it was detected in all samples except for one of the Kanazawa winter samples. The BDE-209 concentration was much higher in Beijing than in Kanazawa. No seasonal variation was found for the BDE-209 concentration in Kanazawa. On the other hand, in Beijing, the BDE-209 concentration was significantly higher in summer than winter. BDE-209 primarily exists in the atmospheric particle phase, irrespective of ambient temperatures (Yang et al., 2012); it is thought, therefore, not to be susceptible to seasonal variation. However, Hu et al. (2011) also reported that concentrations of PBDEs, including BDE-209, were relatively

high in summer. They considered this seasonal variation to be consistent with the seasonal trends in atmospheric suspended particle concentrations in Beijing. We believe that further study is necessary to understand the reason for high BDE-209 concentration during the summer. In Beijing, the concentration of BDE-209 was significantly higher than that of DP, indicating that BDE-209 is still an important flame retardant in China. Yu et al. (2011) also reported the BDE-209 predominance between PBDE and DP in the air particulate samples collected in Shanghai, China. Significant correlations in the winter samples of Kanazawa (summer: R^2= 0.02; winter: R^2 = 0.70, ρ = 0.01) and Beijing (summer: R^2 = 0.56, ρ = 0.05; winter: R^2 = 0.98, ρ < 0.01) were found between the concentrations of ΣDP and BDE-209. This similarity in atmospheric behavior of DP and BDE-209 was assumed to indicate a common end usage and release mechanism for these compounds.

The result of DP particle size fraction distribution behavior is shown in Fig 13. Regardless of the sampling period, the highest DP concentration (~43% of ΣDP) was found in the finest particle group (< 1.1 μm). For the f_{anti} value, there was a tendency for the value to decrease with the decreasing particle size. The results of DP in total suspended particle matter samples indicated that the atmospheric DP in Japanese cities was minimally affected by LRAT, but DP particle size fraction behavior showed the

larger stereo-selective depletion of the *anti*-isomer caused by LRAT in the finer particle stage. The one possible reason for this result is that particles smaller than 2μm are able to remain in ambient air for a longer time and are not as efficiently removed by wet or dry deposition, further prolonging their atmospheric lifetime (Bidleman, 1988; Kurokawa et al., 1998).

2.3.5. Interim summary

We determined the DP concentrations in atmospheric particles in east Asia for samples collected during the same periods in 2010. Relatively high atmospheric DP concentrations were found in several Japanese cities, despite there being no DP manufacturing facility in Japan. Additionally, the DP concentrations were correlated with the population of those cities. These facts indicate that considerable potential sources of atmospheric DP in Japan were released from DP-containing products used in Japan, such as electrical wire, cable coatings, computer connectors, and plastic roofing materials. The atmospheric concentrations of DP and BDE-209 were similar in Kanazawa. However, the BDE-209 abundance in Beijing indicates that BDE-209 is still an important flame retardant in China. In 2006, production of the technical deca-BDE mixture was reported to have reached 15,000 tons in China(Hu et al., 2010).

The highest DP concentration (~43% of ΣDP) was found in the finest particle group (<1.1 μm). There was a tendency for the f_{anti} value to decrease with decreasing particle size. This may contribute to stereo-selective depletion of the anti-isomer caused by long-range atmospheric transport. I therefore conclude that atmospheric DP was released from DP-containing products used in Japan on the whole, but DP in the fine particulates was seen to be partly affected by LRAT.

Table 12 Sampling information

location	Population (million)	season	period	Temperature (ºC)
Sapporo, Japan (latitude43° 04' 54.998", longitude 141° 20' 00.366")	2	summer winter	9—15,Aug 12—18,Feb	25 -5
Sagamihara, Japan (latitude35° 34' 41.675", longitude 139° 23' 21.415")	0.7	summer winter	7—13,Aug 12—17,Feb	27 3
Kanazawa, Japan (latitude36° 32' 51.997", longitude 136° 42' 33.188")	0.5	summer winter	9—16,Aug 11—24,Feb	29 3
Kitakyushu, Japan (latitude33° 53' 43.018", longitude 130° 49' 45.753")	1	summer winter	9—22,Aug 13—19,Feb	29 5
Busan, Korea (latitude35° 15' 16.53", longitude 129° 5' 1.114")	3.6	summer winter	9—15,Aug 12—20,Feb	27 5
Beijing, China (latitude40° 24' 50.94", longitude 116° 43' 3.695")	20	summer winter	9—16,Aug 25—31,Jan	26 -1

Table 13　DP and BDE-209 concentration (pg/m^3) on each sampling site

		syn-DP[a]	*anti*-DP[a]	ΣDP[b]	BDE-209[a]
Sapporo	Summer (n=7)	1.49 ± 1.52	4.36 ± 5.83	5.46 ± 5.51	
	Winter (n=7)	1.31 ± 0.91	3.78 ± 2.51		
Sagamihara	Summer (n=7)	0.47 ± 0.25	1.04 ± 0.55	4.12 ± 3.70	
	Winter (n=7)	1.76 ± 0.95	4.97 ± 2.77		
Kanazawa	Summer (n=7)	0.73 ± 0.45	2.26 ± 1.57	2.37 ± 1.72	1.63 ± 0.81
	Winter (n=7)	0.49 ± 0.33	1.26 ± 0.89		1.81 ± 2.21
Kitakyushu	Summer (n=7)	1.80 ± 3.78	2.68 ± 4.62	3.61 ± 5.91	
	Winter (n=7)	0.63 ± 0.38	2.10 ± 1.47		
Busan	Summer (n=7)	0.26 ± 0.12	0.62 ± 0.32	0.88 ± 0.78	
	Winter (n=7)	0.41 ± 0.59	0.46 ± 0.49		
Beijing	Summer (n=7)	0.41 ± 0.22	0.83 ± 0.73	1.98 ± 3.90	54.3 ± 19.2
	Winter (n=7)	0.76 ± 1.57	1.96 ± 3.99		12.2 ± 12.9

[a] the mean concentration ± standard deviation
[b] the mean total DP concentrations of the all samples on each site ± standard deviation
detection thresholds < 0.01 pg/m^3 for *syn*- and *anti*-DP; < 0.12 pg/m^3 for BDE-209

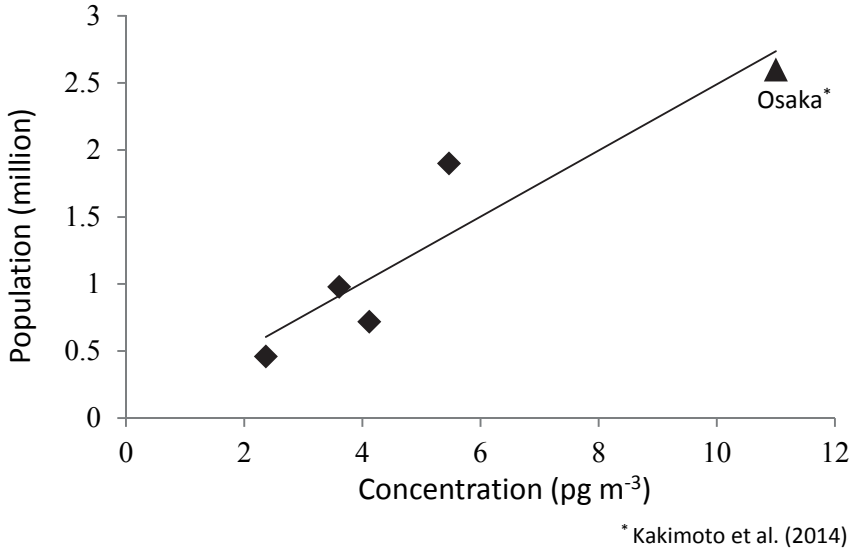


Fig.11 Mean ΣDP concentrations of five cities in Japan against the population

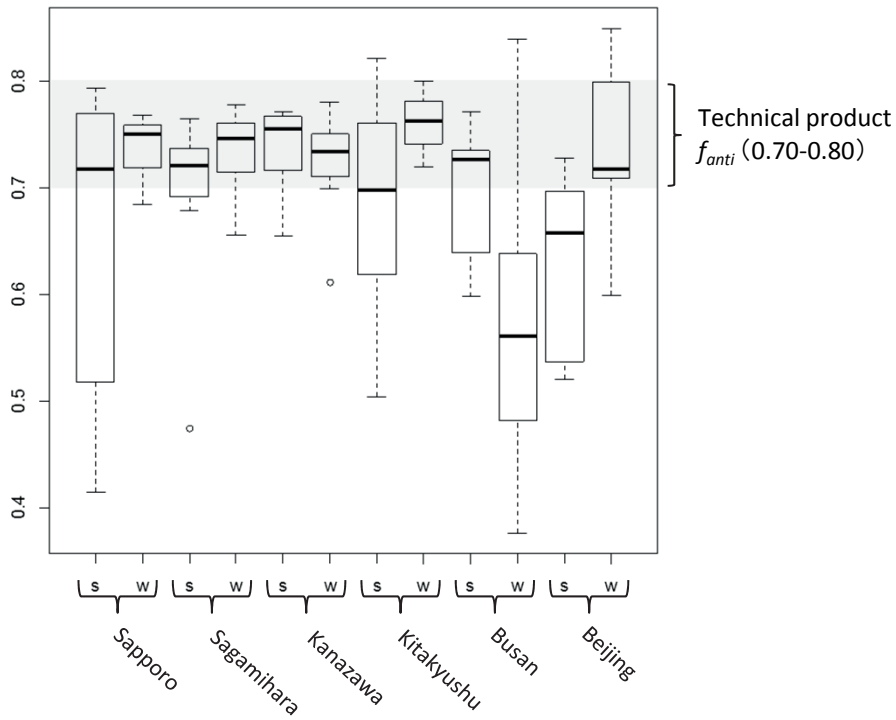


Fig.12 f_{anti} values on each sampling site

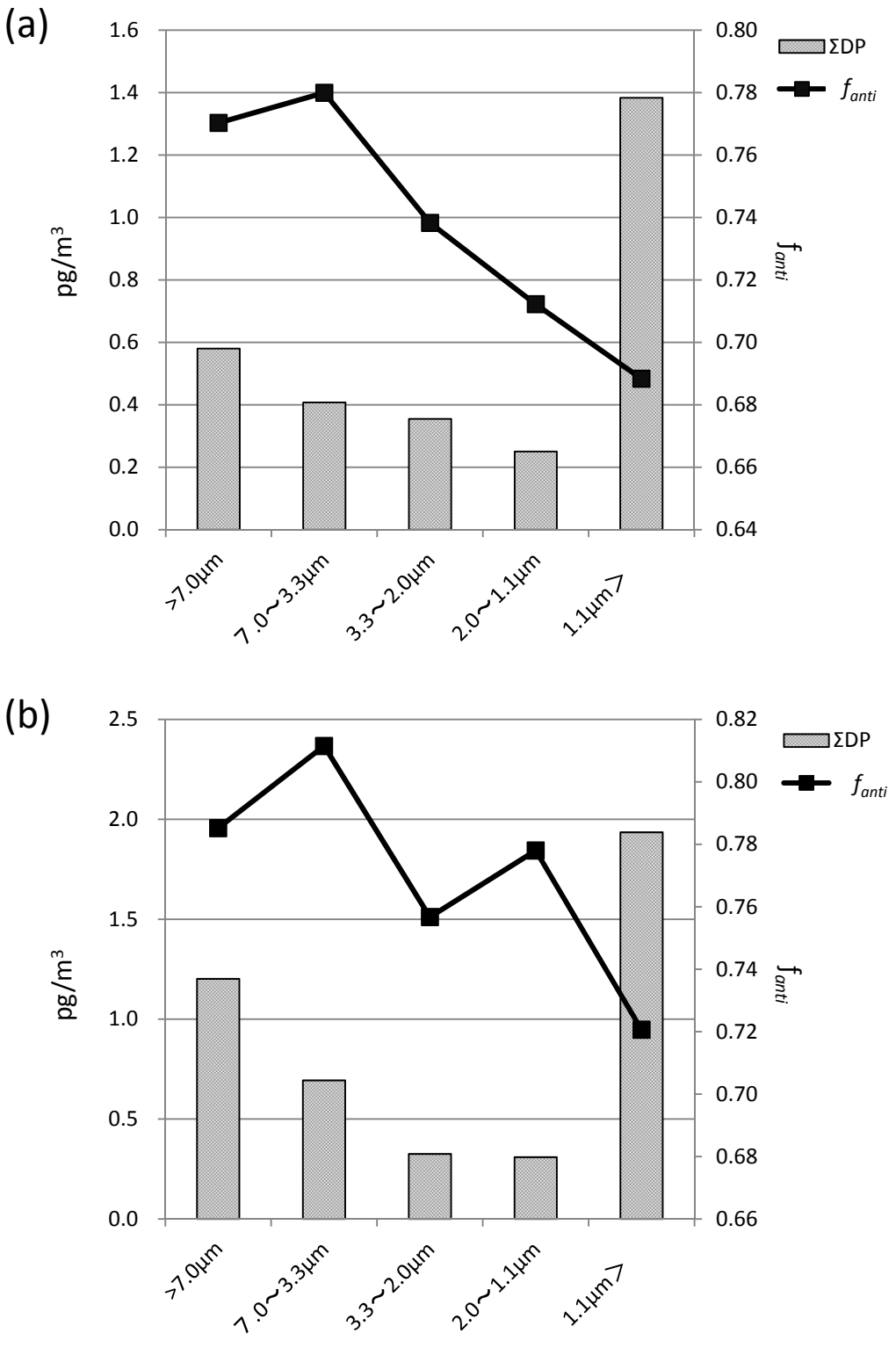


Fig.13 DP concentrations and f_{anti} values on each particle size
(a) 72 h collection (b) 168 h collection

2.4. Atmospheric chlorinated polycyclic aromatic hydrocarbons

2.4.1. Abstract

This study estimates atmospheric concentrations of chlorinated polycyclic aromatic hydrocarbons (ClPAHs) and polycyclic aromatic hydrocarbons (PAHs) in East Asia using a gas chromatograph with a high resolution mass spectrometer (GC-HRMS). ClPAHs are ubiquitously generated from PAHs through substitution, and some ClPAHs show higher aryl hydrocarbon receptor (AhR)-mediated activities than their parent PAHs. Atmospheric particles were collected using a high-volume air sampler equipped with a quartz-fiber filter. We determined the ClPAH concentrations of atmospheric particles collected in Japan (Sapporo, Sagamihara, Kanazawa, and Kitakyushu), Korea (Busan), and China (Beijing). The concentrations of ClPAHs were highest in the winter Beijing sample, where the total mean concentration was approximately 15 to 70 times higher than in the winter samples from Japan and Korea. The concentrations of Σ19ClPAHs and Σ9PAHs were significantly correlated in the Kanazawa and Busan samples. This indicates that within those cities ClPAHs and PAHs share the same origin, implying direct chlorination of parent PAHs. Toxic equivalent concentrations (TEQs) of the total ClPAHs and PAHs were lowest in Kanazawa in the summer, reaching 1.18 and 2,610 fg-TEQ m^{-3} respectively, and highest in Beijing in the winter, reaching 627 and 4,240,000 fg-TEQ m^{-3} respectively.

2.4.2. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are environmental carcinogens and mutagens (IARC, 2013) that are distributed in the environment through combustion, discharge of fossil fuels, and automobile emissions (Takasuga et al., 2007; Pinedo et al., 2013; Lee et al., 1995). Humans are exposed to PAHs from various sources, including certain occupational environments, dietary sources, cigarette smoking, and fossil fuels (Lodovici et al., 1995; Hoffmann and Hoffmann, 1997; Liu et al., 2001). In recent

decades, the detection of chlorinated PAHs (ClPAHs) in the environment, including in tap water, road tunnels, and soil (Shiraishi et al., 1985; Nilsson and Öestman, 1993; Sugiyama et al., 1999; Ishaq et al., 2003) has attracted considerable interest. The structure of ClPAHs is similar to polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs), and polychlorinated biphenyls (PCBs). PAHs generated from the combustion of organic compounds can lead to concurrent production of ClPAHs (Takasuga et al., 2007). Horii et al. (2008) reported a mechanism of direct chlorination of parent PAHs during incineration of wastes. In addition, the combustion of polyvinylchloride (PVC) and plastic wrapping made from polyvinylidene chloride (PVDC) results in the production of ClPAHs (Fujima et al., 2006). The evidence suggests that the combustion of organic materials in combination with a chlorine source, or of organic materials containing chlorine, is a possible source of environmental ClPAH pollution. There are concerns that ClPAHs have adverse effects on humans, based on reports that some ClPAHs exhibited mutagenic potential in *Salmonella* Typhimurium strains TA98 and TA100 (Colmsjö et al., 1984; Bhatia et al., 1987), and showed stronger aryl hydrocarbon receptor (AhR)-mediated activities than those of the parent PAHs in yeast assays (Ohura et al., 2007). Due to the lack of authentic ClPAH analytical standards, their environmental chemistry has not yet been examined in detail.

In this study, we compare levels of atmospheric ClPAH contamination across East Asian countries. Atmospheric particles were collected using a high-volume air sampler equipped with a quartz-fiber filter, and we determined ClPAHs using a gas chromatograph with a high-resolution mass spectrometer (GC-HRMS).

2.4.3. Materials and methods

2.4.3.1. Sample preparation

TSP particulates samples were collected from six cities in Japan, Korea, and China. Sampling information was described in section *2.3.3* and detailed information about the sampling sites and times is presented in Table 12.

2.4.3.2. Chemicals

A non-labeled 19ClPAH (Fig. 8) solution mix was provided by Prof. Ohura (Ohura et al., 2005). A non-labeled PAH solution mix was purchased from Accu Standard (CT, USA). Deuterium-labeled standards (phenanthrene-d_{10}, chrysene-d_{12}, and perylene-d_{12}) were purchased from Wako Pure Chemicals (Osaka, Japan), and pyrene-d_{10} was purchased from Cambridge Isotope Laboratories (MA, USA). Organic solvents of pesticide-analysis grade were used for the extraction and cleanup of samples, and a silica gel of dioxin analysis grade was purchased from Wako Pure Chemicals. The

nineteen different ClPAHs analyzed in this study are abbreviated as follows: 9-chlorophenanthrene (9-ClPhe), 2-chloroanthracene (2-ClAnt), 9-chloroanthracene (9-ClAnt), 3,9-dichlorophenanthrene (3,9-diClPhe), 9,10-dichloroanthracene (9,10-diClAnt), 1,9-dichlorophenanthrene (1,9-diClPhe), 9,10-dichlorophenanthrene (9,10-diClPhe), 3-chlorofluoranthene (3-ClFluor), 8-chlorofluoranthene (8-ClFluor), 1-chloropyrene (1-ClPy), 3,9,10-trichlorophenanthrene (3,9,10-triClPhe), 1,3-dichlorofluoranthene (1,3-diClFluor), 3,8-dichlorofluoranthene (3,8-diClFluor), 3,4-dichlorofluoranthene (3,4-diClFluor), 6-chlorochrysene (6-ClChry), 7-chlorobenz[*a*]anthracene (7-ClBaA), 6,12-dichlorochrysene (6,12-diClChry), 7,12-dichlorobenz[*a*]anthracene (7,12-diClBaA), and 6-chlorobenzo[*a*]pyrene (6-ClBaP). The PAHs used in this study are abbreviated as follows: phenanthrene (Phe), anthracene (Ant), pyrene (Pyr), chrysene (Chry), benz[*a*]anthracene (BaA), benzo[*b*]fluoranthene (BbF), benzo[*k*]fluoranthene (BkF), benzo[*a*]pyrene (BaP), and perylene (Pery).

2.4.3.3. Extraction and cleanup

The treatments for the atmospheric particle samples followed almost the same method used by Kitazawa et al. (2006). Briefly: the filters were treated ultrasonically for 30 min with 50 mL of dichloromethane containing a stable isotope standard mixture

(phenanthrene-d_{10}, pyrene-d_{10}, chrysene-d_{12}, and perylene-d_{12}; 5 ng each). After centrifugation, the supernatant was concentrated to approximately 1 mL. The solution was cleaned using a silica gel column (0.5 g). Then 10 mL of a hexane-dichloromethane mixture (9:1, v/v) was used as an eluent, 0.5 mL of nonane was added to this solution, after which it was evaporated until the hexane was completely removed. Finally, 1 μL of the sample solution was used for analysis by GC-HRMS.

2.4.3.4. GC/MS conditions

GC-HRMS analyses used a gas chromatograph (6890 series, Agilent Technologies, CA, USA) coupled with a high-resolution mass spectrometer (JMS-800D, JOEL, Tokyo, Japan). The GC conditions were as follows: column, capillary column (DB-5MS, 30 m, 0.25 mm i.d., 0.25 μm film thickness, J&W Scientific, CA, USA); column temperature program: 100°C (held for 1 min) to 200°C at 25°C min^{-1}, then to 310°C (held for 5 min) at 5°C min^{-1}; carrier gas, helium; column head pressure, 10 psi; injection temperature, 270°C; injection mode, splitless (splitless time, 1.5 min). The MS conditions were as follows: electron ionization mode; electron energy, 38 eV; filament current, 500 μA; ion source temperature, 270°C; resolution, 10,000 (10% valley definition). The monitoring ions for each target compound are shown in Table 14.

2.4.3.5. Quality assurance and quality control

The filters were spiked with the working solution, which includes all 19 ClPAHs and 9 PAHs, then extracted and analyzed by the same method used for the samples. Recoveries of each ClPAH and PAH in the filter samples (n = 5) all exceeded 90%, and the relative standard deviations of the recoveries of each ClPAH and PAH were less than 16.8% (spiking amounts: ClPAHs, 2–4.4 ng; PAHs, 20 ng) (Table 15). The calculated detection limits for ClPAHs and PAHs were 0.05–0.39 pg m^{-3} and 0.28–1.43 pg m^{-3} respectively (*S*/*N* = 3). The ClPAH and PAH congeners were identified by comparing their relative retention times, and by monitoring ion ratios with those of authentic calibration standards. A deviation of the ion intensity ratio within 20% of the mean value of the calibration standard was considered acceptable. The quality control standards were measured after every 10 samples to monitor instrumental drift. For each batch of samples a procedural blank was performed, none of which detected ClPAHs or PAHs.

2.4.4. Results and discussion

2.4.4.1. ClPAHs and PAHs in atmospheric particulates

The mean concentrations, concentration ranges, and detection rates of the ClPAHs and their parent PAHs are shown in Table 16. Irrespective of the season, both ClPAHs and

PAHs were highest in Beijing, China. This trend is similar to that found by a previous study that analyzed PAHs and NPAHs in China, Korea, Russia, and Japan(Tang et al., 2005). Both ClPAHs and PAHs showed higher concentrations in winter than in summer, regardless of the sampling site. These results are in agreement with Ohura et al. (2013), who reported that atmospheric gaseous and particulate ClPAH concentrations increased significantly in winter. Kitazawa et al. (2006) also reported that ClPAH and PAH concentrations in atmospheric particles increased in the cold season and decreased in the warm season. Possible causes for these seasonal trends include: (i) greater emission of particulates from fossil fuel-burning heaters in winter, (ii) gas–particle distributions, and (iii) photochemical degradation in summer. However, the atmospheric behavior of ClPAHs is more complicated than that of PAHs for the following reasons. Firstly, with regard to, gas–particle partitioning, the partitioning of PAHs into particle phase showed a dependency on molecular weight, whereas that of ClPAHs does not completely follow this trend (Ohura et al., 2013). Fu and Suuberg (2012) reported decreased vapor pressure accompanying chlorination of the parent PAH (3 to 4 rings). On the other hand, Ohura et al. (2008) reported that among the ClPhe congeners, the number of chlorines substituted on the Phe does not affect gas–particle partitioning. Secondly, in general, ClPAHs are generated from the combustion of organic materials in combination with

the chlorine source, or of organic materials containing chlorine. However, there is also the possibility of chlorination of PAHs on the surface of ambient particles (Ohura et al., 2013), and ClPAH production via photochemical reaction of PAH present in tidal flats (Sankoda et al., 2012).

Among the analyzed ClPAHs, 1-ClPyr was detected in all samples, and 6-ClBaP was also ubiquitous, except in the summer samples from Kitakyushu. The high concentrations of 1-ClPyr and 6-ClBaP among the ClPAHs in our study is in agreement with the profiles reported from air collected at Shizuoka, Japan (Kitazawa et al., 2006), in fly ash from incineration facilities (Horii et al., 2008), and in air collected from a road tunnel (high traffic areas) (Nilsson and Öestman, 1993).

The concentration ratios of 1-ClPyr and 6-ClBaP normalized to their corresponding parent PAHs for each sampling site are summarized in Fig. 14 as box plots, which were made using R statistical software version 3.0.3. This figure indicates that the chlorination of PAHs proceeds to a greater extent in Japan and Korea, compared to China, especially in winter. It is reported that the ClPAH/PAH ratio in ash varied according to the type of waste incinerator (Horii et al., 2008), and that the formation of chlorinated hydrocarbons and PAHs was affected by parameters including furnace temperature and the types of material combusted (Wang et al., 2003; Takasuga et al.,

2007). Consequently, we consider that the lower ClPAH/PAH ratio in China is influenced by the contributions from specific PAH emission sources, which generate relatively low ClPAHs. The 1-ClPyr/Pyr ratio increased in winter except in Beijing, and that of 6-ClBaP/BaP decreased in winter, except at Kanazawa. The compositions of nine ClPAHs and seven PAHs are shown in Fig. 15. At all locations, the proportions of 6-ClBaP and BaP were enhanced in summer samples compared with winter samples. Ohura et al. (2013) also reported that among ClPAHs, 6-ClBaP has low vapor pressure and is a significant contributor in the particulate phase, and that the contribution of 6-ClBaP was greater in summer than in winter. On the other hand, the contributions of ClPhe, ClFluor, and their parent PAHs were enhanced in the winter samples from every sampling site. This is thought, to some extent, to be a result of the temperature-dependent differences in the gas-particle partitioning of ClPAHs in the atmosphere, especially for semi-volatile ClPAHs.

The concentrations of Σ19ClPAHs and Σ9PAHs (Fig. 16) showed significant correlations within the Kanazawa samples (summer: $r = 0.93$, $\rho < 0.01$; and winter: $r = 0.94$, $\rho < 0.01$) and Busan samples (summer: $r = 0.98$, $\rho < 0.01$; and winter: $r = 0.96$, $\rho < 0.01$). There were the seasonal correlations within the Sapporo winter samples ($r = 0.86$, $\rho < 0.01$) and the Kitakyushu summer samples ($r = 0.90$, $\rho < 0.01$). These results

indicate that Kanazawa and Busan have uninterrupted, non-seasonal sources of ClPAHs formed through direct chlorination of the parent PAHs, for example from incineration facilities, possibly via a previously reported mechanism (Horii et al., 2008). Conversely, ClPAHs in Beijing are thought to be derived from more complex sources, including secondary generation sources such as photochemical reaction and automobile exhaust fumes (Nilsson and Öestman, 1993), or from PAH-generating sources that result in the irregular generation of ClPAHs.

2.4.4.2. Toxicity evaluation of ClPAHs and PAHs in atmospheric particulates

Ohura et al. (2007; 2009) calculated toxic equivalent concentrations (TEQs) for mixtures of ClPAHs and PAHs in air particulates using the following equation:

$$TEQ = \Sigma [C_i] \times REP_{BaP,i}/60$$

where C_i represents the mean concentration of an individual ClPAH or PAH in air particulates in this study, and REP_{BaP} represents the degree of toxicity compared to BaP as determined by Ohura et al. (2007; 2009) for AhR-mediated activities of ClPAHs in a yeast assay system (Table 17). In the YCM3 cell assay system, the intensity of the AhR-associated toxicity of TCDD was 60 times that of BaP (Kawanishi et al., 2003). On the basis of these relative potency values, we estimated TEQs for Σ17ClPAHs and Σ7PAHs using the individual REP_{BaP} values and corresponding mean concentrations in

the air particulates studied (Table 18). Ohura et al. (2009) reported that the TEQs of total ClPAHs and PAHs in Shizuoka, Japan were 44.1 fg-TEQ m^{-3} and 11,500 fg-TEQ m^{-3} respectively, which are similar to those found in Sagamihara, Japan in this study. Assuming that an average person stays outdoors all day, and that the mean inhalation rate for an adult is 15.7 m^3 day^{-1} (US EPA, 2011), we estimated the mean daily TEQ exposure to ClPAHs and PAHs: the lowest values are 18.5 fg-TEQ day^{-1} and 41,000 fg-TEQ day^{-1} respectively, recorded in summer in Kanazawa, whereas the highest are 9,840 fg-TEQ day^{-1} and 66,600,000 fg-TEQ day^{-1} respectively, recorded in winter in Beijing. The contributions of ClPAHs to the total TEQs of ClPAHs and PAHs were very low, and were highest in the summer Sapporo samples (0.23%) and lowest in winter Beijing samples (0.01%). However, as human exposures to ClPAHs remain incompletely defined, further studies should seek to elucidate the relevant exposure pathways.

2.4.5. Interim summary

There is presently limited information on atmospheric ClPAH concentrations (Ohura et al., 2004; Ohura et al., 2008; Ohura et al., 2009). This is the first multinational comparison of seasonal atmospheric ClPAH concentrations. Among the study locations,

Beijing showed the highest ClPAH concentrations, especially in winter, and a lower ClPAH-PAH ratio than at any other site. We identified differences in the correlativity between ClPAHs and PAHs within the sampling sites.

Table 14
Monitoring ions for ClPAHs and PAHs used in this study

Compounds	Monitor ions (*m/z*)		Surrogate I.S.[a]
	Quantification	Qualification	
9-ClPhe	212.0393	214.0363	Phe-d_{10}
2-ClAnt	212.0393	214.0363	Phe-d_{10}
9-ClAnt	212.0393	214.0363	Phe-d_{10}
3,9-diClPhe	246.0003	247.9973	Pyr-d_{10}
9,10-diClAnt	246.0003	247.9973	Pyr-d_{10}
1,9-diClPhe	246.0003	247.9973	Pyr-d_{10}
9,10-diClPhe	246.0003	247.9973	Pyr-d_{10}
3-ClFluor	236.0393	238.0363	Pyr-d_{10}
8-ClFluor	236.0393	238.0363	Pyr-d_{10}
1-ClPyr	236.0393	238.0363	Pyr-d_{10}
3,9,10-triClPhe	279.9613	281.9583	Pyr-d_{10}
1,3-diClFluor	270.0003	271.9973	Chry-d_{12}
3,8-diClFluor	270.0003	271.9973	Chry-d_{12}
3,4-diClFluor	270.0003	271.9973	Chry-d_{12}
6-ClChry	262.0549	264.0519	Chry-d_{12}
7-ClBaA	262.0549	264.0519	Chry-d_{12}
6,12-diClChry	296.0160	298.0130	Chry-d_{12}
7,12-diClBaA	296.0160	298.0130	Chry-d_{12}
6-ClBaP	286.0549	288.0519	Pery-d_{12}
Phe	178.0783	176.0626	Phe-d_{10}
Ant	178.0783	176.0626	Phe-d_{10}
Fluor	202.0783	200.0626	Pyr-d_{10}
Pyr	202.0783	200.0626	Pyr-d_{10}
Chry	228.0939	226.0782	Chry-d_{12}
BaA	228.0939	226.0782	Chry-d_{12}
BbF	252.0939	250.0782	Pery-d_{12}
BkF	252.0939	250.0782	Pery-d_{12}
BaP	252.0939	250.0782	Pery-d_{12}
Phe-d_{10}	188.1410	184.1159	-
Pyr-d_{10}	212.1410	208.1159	-
Chry-d_{12}	240.1692	236.1410	-
Pery-d_{12}	264.1692	260.1441	-

[a] Surrogate internal standard used for quantification of each native compounds

Table 15 Results of recovery tests of ClPAHs and PAHs

	spike amount (ng)	recovery(%)	R.S.D.(%)
ClPAHs			
9-ClPhe	2.14	201%	3.0
2-ClAnt	4.4	472%	6.2
9-ClAnt	2.1	387%	16.8
3,9-diClPhe	2.56	102%	3.5
9,10-diClAnt &1,9-diClPhe	2.4	107%	4.7
9,10-diClPhe	2.16	101%	6.8
3-ClFluor	2.06	91%	1.7
8-ClFluor	2.06	114%	2.8
1-ClPyr	2.2	102%	2.8
3,9,10-triClPhe	4.2	148%	4.6
1,3-diClFluor	4.16	120%	9.7
3,8-diClFluor	4.08	110%	6.1
3,4-diClFluor	4.04	113%	5.4
6-ClChry	2.06	95%	2.2
7-ClBaA	2	106%	2.4
6,12-diClChry	2.42	105%	4.4
7,12-diClBaA	2.18	114%	2.8
6-ClBaP	2.2	107%	2.4
PAHs			
Phe	20	97%	4.9
Ant	20	96%	3.5
Fluor	20	96%	4.7
Pyr	20	96%	3.5
Chry	20	104%	2.8
BaA	20	114%	5.8
BbF	20	94%	3.3
BkF	20	103%	4.5
BaP	20	94%	6.0

Table 16 Mean atmospheric particulate concentrations (pg m^{-3}) of ClPAHs and PAHs

	LOD	Sapporo (Japan)				Sagamihara (Japan)				Kanazawa (Japan)				Kita-kyushu (Japan)			
		summer(n=7)		winter(n=7)		summer(n=7)		winter(n=6)		summer(n=7)		winter(n=7)		summer(n=7)		winter(n=7)	
ClPAHs																	
9-ClPhe	0.28	0[a]	<0.28[b]	100	1.23 (0.40-2.07)	0	<0.28	100	1.19 (0.66-1.93)	0[a]	<0.28[b]	0	<0.28	43	0.17 (<0.28-0.46)	100	1.27 (0.69-1.74)
2-ClAnt	0.39	0	<0.39	29	0.18 (<0.39-0.74)	0	<0.39	0	<0.39	0	<0.39	0	<0.39	0	<0.39	43	0.20 (<0.39-0.58)
9-ClAnt	0.36	0	<0.36	0	<0.36	0	<0.36	0	<0.36	0	<0.36	0	<0.36	0	<0.36	0	<0.36
3,9-diClPhe	0.08	0	<0.08	0	<0.08	0	<0.08	0	<0.08	0	<0.08	0	<0.08	0	<0.08	0	<0.08
9,10-diClAnt &1,9-diClPhe	0.08	0	<0.08	0	<0.08	0	<0.08	0	<0.08	0	<0.08	0	<0.08	0	<0.08	0	<0.08
9,10-diClPhe	0.11	0	<0.11	0	<0.11	0	<0.11	0	<0.11	0	<0.11	0	<0.11	0	<0.11	0	<0.11
3-ClFluor	0.06	100	0.29 (0.14-0.43)	100	1.99 (1.16-3.54)	100	0.16 (0.07-0.23)	100	1.03 (0.66-1.48)	0	<0.06	100	0.57 (0.17-1.10)	100	0.24 (0.14-0.61)	100	2.17 (0.79-4.66)
8-ClFluor	0.10	0	<0.10	100	0.38 (0.18-0.65)	0	<0.10	100	0.21 (0.11-0.34)	0	<0.10	43	0.06 (<0.10-0.17)	0	<0.10	100	0.41 (0.14-0.68)
1-ClPyr	0.05	100	0.26 (0.08-0.48)	100	3.17 (1.21-6.18)	100	0.31 (0.08-1.04)	100	3.28 (1.52-8.08)	100	0.19 (0.09-0.28)	100	1.65 (0.32-4.11)	100	0.31 (0.09-0.96)	100	5.56 (2.90-11.8)
3,9,10-triClPhe	0.17	0	<0.17	0	<0.17	0	<0.17	0	<0.17	0	<0.17	0	<0.17	0	<0.17	0	<0.17
1,3-diClFluor	0.14	0	<0.14	0	<0.14	0	<0.14	0	<0.14	0	<0.14	0	<0.14	0	<0.14	0	<0.14
3,8-diClFluor	0.11	0	<0.11	0	<0.11	0	<0.11	0	<0.11	0	<0.11	0	<0.11	0	<0.11	0	<0.11
3,4-diClFluor	0.15	0	<0.15	0	<0.15	0	<0.15	0	<0.15	0	<0.15	0	<0.15	0	<0.15	100	1.00 (0.37-1.81)
6-ClChry	0.10	71	0.18 (<0.10-0.38)	100	0.34 (0.18-0.65)	71	0.11 (<0.10-0.21)	100	0.59 (0.36-0.75)	0	<0.10	0	<0.10	29	0.07 (<0.10-0.35)	100	0.69 (0.26-1.75)
7-ClBaA	0.10	43	0.06 (<0.10-0.19)	100	0.26 (0.16-0.37)	57	0.10 (<0.10-0.26)	100	0.76 (0.35-1.09)	0	<0.10	0	<0.10	29	0.06 (<0.10-0.31)	100	0.59 (0.20-1.09)
6,12-diClChry	0.12	0	<0.12	0	<0.12	0	<0.12	0	<0.12	0	<0.12	0	<0.12	0	<0.12	0	<0.12
7,12-diClBaA	0.10	0	<0.10	0	<0.10	0	<0.10	0	<0.10	0	<0.10	0	<0.10	0	<0.10	0	<0.10
6-ClBaP	0.06	100	0.48 (0.12-0.86)	100	0.97 (0.52-1.78)	100	0.87 (0.14-3.01)	100	1.38 (1.00-1.67)	100	0.57 (0.21-1.25)	100	1.01 (0.22-2.71)	86	0.53 (<0.06-1.50)	100	3.44 (1.53-7.79)
Σ19ClPAHs			1.28		8.51		1.54		8.43		0.76		3.29		1.38		14.3
PAHs																	
Phe	0.98		31.1 (17.4-62.1)		450 (126-1,000)		27.9 (10.9-95)		154 (103-217)		13.4 (5.6-19.1)		139 (31.2-321)		39.4 (13.8-142)		587 (344-946)
Ant	0.49		4.0 (3.1-6.8)		16.1 (5.3-27.7)		6.2 (3.4-17.3)		10.3 (7.1-12.9)		5.1 (4.1-6.1)		8.6 (4.7-15.9)		7.9 (2.7-31.4)		53.5 (10.7-188)
Fluor	0.45		44.1 (21.9-69.3)		801 (237-1,333)		61.6 (14.2-248)		287 (165-404)		35.5 (17.2-61)		173 (35.2-386)		62.1 (17.3-189)		945 (515-1,914)
Pyr	0.46		43.0 (17.9-74.6)		573 (136-1,040)		55.5 (13.4-221)		233 (130-322)		34.7 (15.7-54.5)		116 (24.7-246)		53.5 (13.8-165)		725 (338-1,863)
Chry	0.45		52.4 (19.2-137)		362 (99.3-538)		74 (15.0-263)		222 (128-281)		20.6 (10.8-30.7)		27.7 (13.7-49.6)		58.5 (11.1-199)		668 (181-2,216)
BaA	1.43		51.8 (22.7-107)		535 (205-759)		80.9 (18.0-287)		363 (268-415)		43.8 (20.4-76.4)		121 (63.6-213)		68.7 (13.8-191)		910 (278-2,387)
BbF	1.26		60.5 (23.7-116)		306 (144-491)		93.4 (25.8-283)		277 (201-320)		57.3 (40-86.4)		100 (57.3-163)		92.9 (24.9-323)		821 (266-2,448)
BkF	0.28		58.5 (23.8-129)		341 (156-443)		92.4 (28.5-272)		305 (211-388)		44.3 (30.1-69.1)		83.9 (42.0-153)		85.0 (26.0-282)		662 (210-1,989)
BaP	0.60		32.5 (14.1-80.4)		162 (58.4-216)		67.1 (15.2-226)		133 (71.8-196)		41.3 (22.8-63.4)		40.5 (15.0-75.7)		58.6 (11.6-211)		486 (129-1,749)
Σ9PAHs			378		3546		559		1,984		296		811		527		5,857

	LOD	Busan (Korea)				Beijing (China)			
		summer(n=7)		winter(n=7)		summer(n=7)		winter(n=7)	
ClPAHs									
9-ClPhe	0.28	0	<0.28	100	1.86 (0.39-3.10)	43	0.59 (<0.28-1.40)	100	48.0 (29.51-79.97)
2-ClAnt	0.39	0	<0.39	71	0.52 (<0.39-0.87)	0	<0.39	100	28.7 (12.37-55.10)
9-ClAnt	0.36	0	<0.36	0	<0.36	0	<0.36	100	9.05 (4.17-13.78)
3,9-diClPhe	0.08	0	<0.08	0	<0.08	0	<0.08	0	<0.08
9,10-diClAnt &1,9-diClPhe	0.08	0	<0.08	0	<0.08	0	<0.08	0	<0.08
9,10-diClPhe	0.11	0	<0.11	0	<0.11	0	<0.11	0	<0.11
3-ClFluor	0.06	0	0.10 (0.06-0.14)	100	2.65 (0.57-4.14)	100	0.64 (0.28-1.35)	100	27.1 (14.50-57.85)
8-ClFluor	0.10	0	0.01 (<0.10-0.10)	100	0.60 (0.20-1.09)	0	<0.09	100	9.95 (5.00-21.00)
1-ClPyr	0.05	100	0.36 (0.18-0.76)	100	5.24 (1.10-8.68)	100	3.22 (1.17-6.42)	100	56.8 (35.24-126.16)
3,9,10-triClPhe	0.17	0	<0.17	0	<0.17	0	<0.17	0	<0.17
1,3-diClFluor	0.14	0	<0.14	0	<0.14	0	<0.14	0	<0.14
3,8-diClFluor	0.11	0	<0.11	0	<0.11	0	<0.11	0	<0.11
3,4-diClFluor	0.15	0	<0.15	0	<0.15	0	<0.15	0	<0.15
6-ClChry	0.10	29	0.04 (<0.10-0.17)	100	0.59 (0.17-1.00)	71	0.32(<0.10-0.64)	100	6.49 (2.54-12.25)
7-ClBaA	0.10	0	<0.10	100	0.43 (0.17-0.65)	71	0.29 (<0.10-0.52)	100	6.81 (3.84-11.89)
6,12-diClChry	0.12	0	<0.12	0	<0.12	0	<0.12	0	<0.12
7,12-diClBaA	0.10	0	<0.10	0	<0.10	0	<0.10	0	<0.10
6-ClBaP	0.06	100	0.66 (0.22-1.83)	100	2.27 (0.73-3.69)	100	7.70 (2.38-12.0)	100	18.7 (7.28-47.50)
Σ19ClPAHs			1.17		14.2		12.76		211.6
PAHs									
Phe	0.98		25.3 (18.2-40.8)		457 (122-727)		549 (436-822)		47075 (19,763-83,474)
Ant	0.49		5.1 (4.1-6.8)		15.3 (6.1-26.4)		76.9 (67.6-104)		13338 (3,882-33,232)
Fluor	0.45		49.5 (26.2-90.9)		757 (219-1,160)		907 (673-1,240)		90411 (56,862-129,805)
Pyr	0.46		47.7 (26.8-81.3)		560 (180-868)		916 (633-1,240)		85036 (51,192-129,334)
Chry	0.45		47.5 (27.6-91.1)		374 (131-606)		2,254 (634-5,440)		70610 (41,012-129,377)
BaA	1.43		65.3 (36.0-120)		519 (261-772)		1,369 (808-2,540)		35078 (21,685-62,373)
BbF	1.26		62.7 (31.2-113)		364 (171-558)		2,029 (1100-3,910)		27871 (8,259-67,986)
BkF	0.28		61.4 (34.0-109)		349 (196-504)		2,193 (1050-4,250)		27621 (8,966-63,514)
BaP	0.60		40.8 (20.8-76.1)		201 (75.9-291)		1,647 (616-3,220)		23299 (6,786-53,568)
Σ9PAHs			405		3,596		11,940		420,340

[a]Detection rates (%), [b]Mean concentration (concentration range)

In the case of the concentration was under the detection limit, it was assumed to be 0.

Table 17
REP values of each ClPAH relative to BaP used in this study[a]

	REP_{BaP}
ClPAHs	
9-ClPhe	0.03
2-ClAnt	0.10
9-ClAnt	0.03
3,9-diClPhe	0.32
9,10-diClAnt	0.20
1,9-diClPhe	0.12
9,10-diClPhe	0.16
3-ClFluor	0.17
8-ClFluor	0.18
1-ClPyr	0.10
3,9,10-triClPhe	0.77
3,8-diClFluor	5.7
6-ClChry	2.1
7-ClBaA	0.83
6,12-diClChry	0.03
7,12-diClBaA	0.10
6-ClBaP	0.09
PAHs	
Phe	0.004
Ant	0.01
Fluor	0.01
Pyr	0.05
Chry	2.5
BaA	1.4
BaP	1

[a] Ohura et al., 2007 & Ohura et al., 2009

Table 18 Toxic equivalent concentrations (TEQs) of ClPAHs[a] and PAHs[b] (fg-TEQ m^{-3})

	Sapporo		Sagamihara		Kanazawa	
	summer	winter	summer	winter	summer	winter
Σ17ClPAHs	9.05	29.9	7.39	43.0	1.18	6.06
Σ7PAHs	3,980	30,900	6,150	20,200	2,610	4,800
	Kitakyushu		Busan		Beijing	
	summer	winter	summer	winter	summer	winter
Σ17ClPAHs	5.41	55.0	3.29	50.0	34.4	627
Σ7PAHs	5,080	58,000	4,230	31,700	154,000	4,240,000

[a]Σ17ClPAHs reflects the sum of 9-ClPhe, 2-ClAnt, 9-ClAnt, 3,9-diClPhe, 9,10-diClAnt &1,9-diClPhe(average REP_{BaP}), 9,10-diClPhe, 3-ClFluor, 8-ClFluor, 1-ClPyr, 3,9,10-triClPhe, 3,8-diClFluor, 6-ClChry, 7-ClBaA. 6,12-diClChry, 7,12-diClBaA, and 6-ClBaP.
[b]Σ7PAHs reflects the sum of Phe, Ant, Fluor, Pyr, Chry, BaA, and BaP.

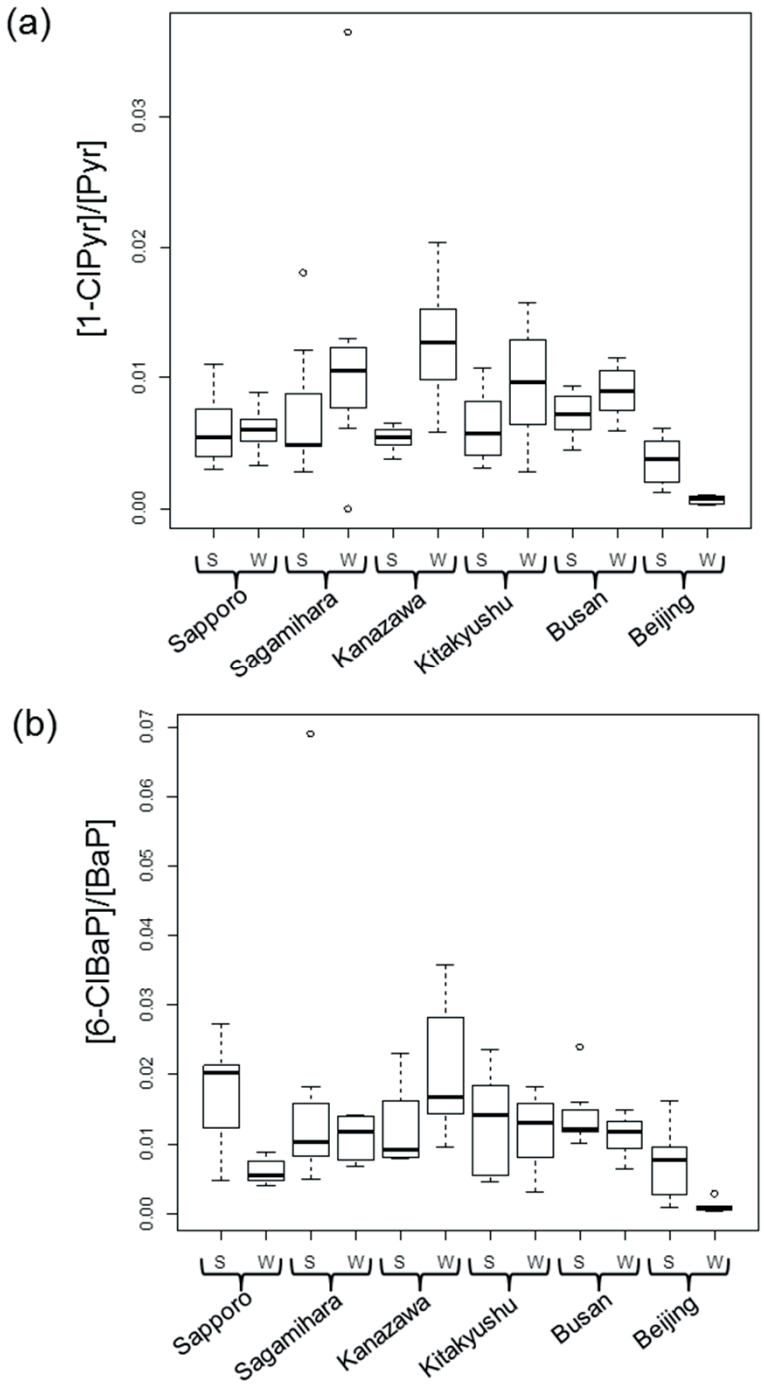


Fig.14 Concentration ratios of 1-ClPyr (a) and 6-ClBaP (b) normalized to corresponding parent PAHs.
(ratio was not calculated where a compound was not detected)

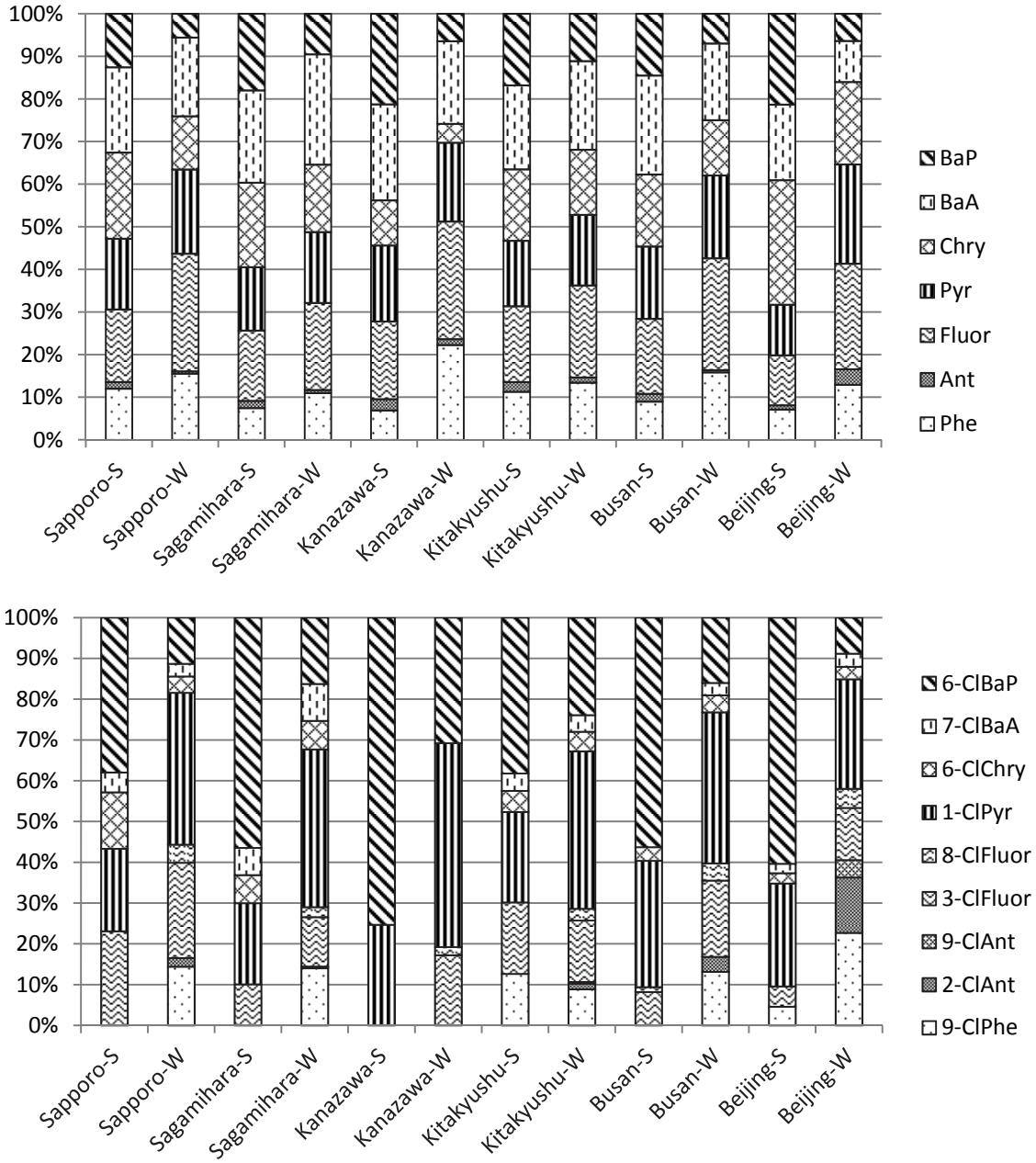


Fig.15 Mean compositions of Σ9ClPAHs and Σ7PAHs

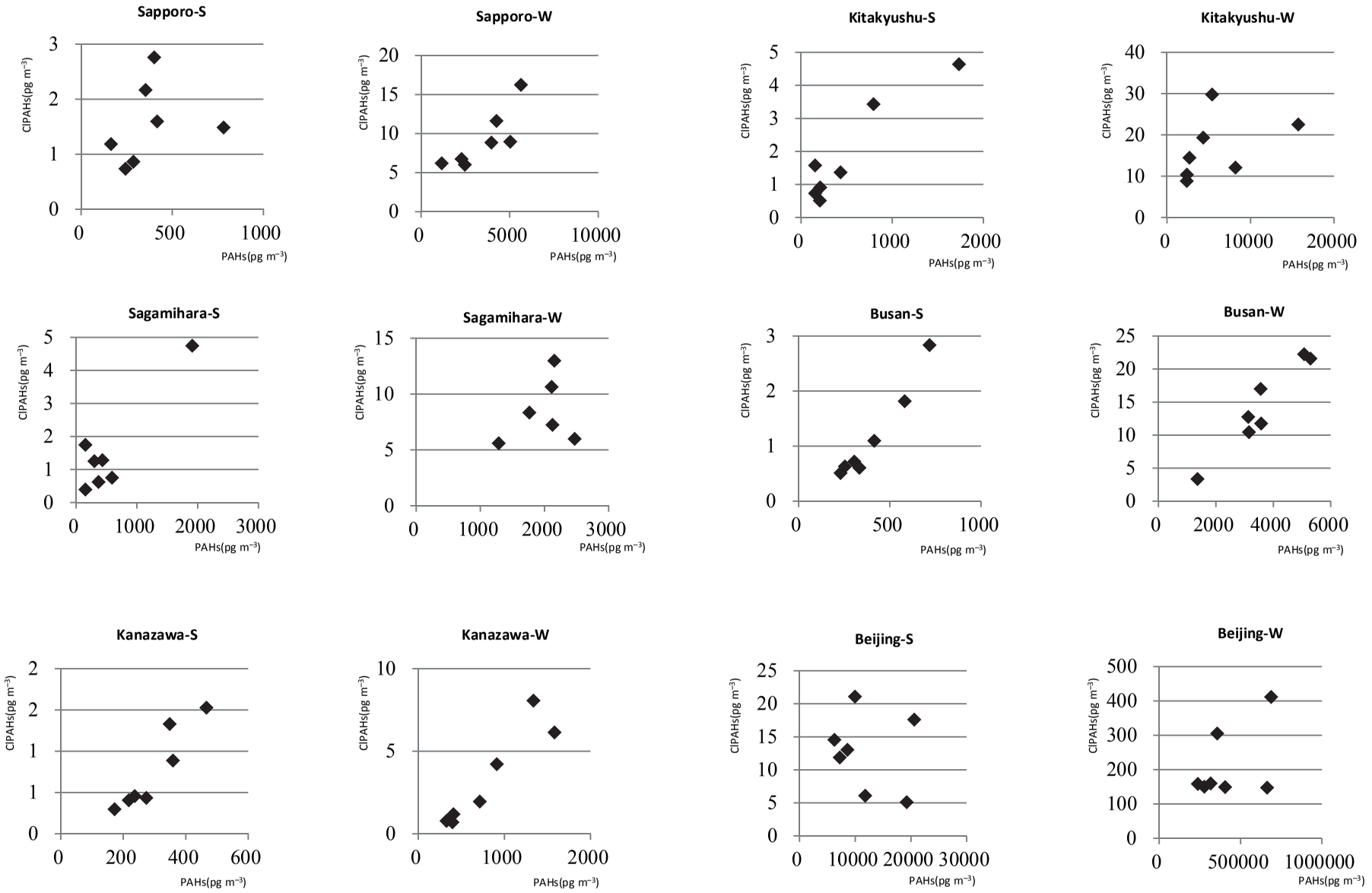


Fig.16 Correlations between the concentrations of Σ19ClPAHs and Σ9PAHs

2.5. Identification and characterization of oxidative metabolites of 1-chloropyrene

2.5.1. Abstract

Polycyclic aromatic hydrocarbons (PAHs) and chlorinated PAHs (ClPAHs) are ubiquitous contaminants that bind the aryl hydrocarbon receptor (AhR) and exhibit mutagenic potential. It is difficult to monitor human exposure levels to ClPAHs because the exposure routes are complicated and environmental concentrations are not always correlated with the levels of PAHs. Urinary PAH metabolites are useful biomarkers for evaluating PAH exposure, and ClPAH metabolites may therefore contribute to the estimation of ClPAH exposure. One of the most abundant ClPAHs present in the environment is 1-chloropyrene (ClPyr) and urinary ClPyr metabolites may have the potential to be good biomarkers to evaluate the level of exposure to ClPAHs. Since the metabolic pathways involving ClPAHs, are still undetermined, we investigated the effect of human cytochrome P450 enzymes on ClPyr and identified three oxidative metabolites by liquid chromatography-tandem mass spectrometry and nuclear magnetic resonance. We found that ClPyr was metabolized most efficiently by the P450 1A1 enzyme, followed by the 1B1 and 1A2 enzymes. Like ClPyr, these metabolites were shown to have agonist activity for the human AhR. We detected these metabolites when ClPyr reacted with a pooled human liver S9 fraction as well as in human urine samples. These results suggest that the metabolites may be used as biomarkers to evaluate the extent of exposure to ClPAHs.

2.5.2. Introduction

It is, of course, important to know the pollution level in the environmental matrix, but it is difficult to analyze every environmental matrix. As described in the former section, atmospheric ClPAH concentrations do not always correlate with the recorded levels of PAHs. This may be because the sources of ClPAHs are thought to be more complex than for PAHs, including secondary generation by photochemical reactions and automobile

exhaust fumes. Alternatively, sources that generate PAHs may generate irregular quantities of ClPAHs. At present, there is still a limited amount of information about the mechanisms of formation (Shiraishi et al., 1985; Wang et al., 2003; Takasuga et al., 2007; Sankoda et al., 2012), environmental pollution levels (Ohura et al., 2013; Ma et al., 2013), biological matrix levels, and human exposure levels to ClPAHs. Since it is difficult to analyze the total intake of contaminants from every source, the measurement of PAH metabolites, which are excreted in the urine, is a well-established method for the estimation of the levels of human PAH exposure (Buchet et al., 1992; Chetiyanukornkul et al., 2002; Kakimoto et al., 2008). It is possible that this approach could be adapted to estimate the level of exposure to ClPAHs, but the pathways involved in the metabolism of ClPAHs are unknown. The most abundant ClPAH found in the environment is 1-chloropyrene (ClPyr) (Ohura et al., 2008; Kakimoto et al., 2014; Ma et al., 2009) and therefore this study investigated ClPyr metabolites as potential biomarkers. We studied the metabolic behavior of ClPyr by searching for phase I metabolites *in vitro* using cytochrome P450. We synthesized the metabolites, identified their chemical structures by nuclear magnetic resonance (NMR) analysis, and determined the kinetic parameters of the cytochrome P450 enzymes (P450) for ClPyr. P450 enzymes play a key role in the initial step of xenobiotic detoxification but, as a

result, can also produce highly mutagenic and carcinogenic metabolites such as benzo[*a*]pyrene (B[*a*]P). We evaluated the activities of ClPyr metabolites as ligands for the AhR in order to assess their potential risk for human health.

2.5.3. Materials and methods

2.5.3.1. Chemicals and reagents

1-chloropyrene was purchased from Frinton Laboratories (NJ, USA). 1-hydroxy pyrene and organic solvents for pesticide analysis, high-performance liquid chromatography (LC), and NMR were purchased from Wako Pure Chemicals (Osaka, Japan). NADPH, glucose-6-phosphate (G6P), and G6P dehydrogenase (G6PD) were purchased from Oriental Yeast (Tokyo, Japan). Pooled human liver S9 and human P450 recombinant microsomes were obtained from Corning Incorporated Life Sciences (MA, USA). The *Saccharomyces cerevisiae* strain, YCM3, expressing human AhR and AhR nuclear translocator (Arnt), carrying a lacZ reporter plasmid containing the aryl hydrocarbon response element (XRE) system, was kindly provided by Dr. C. A. Miller, III (Tulane University, LA, USA)(Miller 1999).

2.5.3.2. P450 enzyme assay

A reaction mixture containing ClPyr, 1.3 mM NADPH, 3.3 mM G6P, 0.4 U mL^{-1} G6PD, and 3.3 mM magnesium chloride in 100 mM potassium phosphate (pH 7.4) was pre-incubated at 37°C and the enzymatic reaction was initiated by addition of recombinant P450 enzyme. After an appropriate incubation at 37°C, the reaction was terminated by the addition of an equal volume of ice-cold acetonitrile. The mixture was vortexed vigorously and centrifuged at 10,000 g for 10 min. After centrifugation, the mixture was incubated at −20°C for 2 h and then the upper acetonitrile layer was collected; an aliquot of this layer was analyzed by liquid chromatography. To determine the kinetic parameters, P450 enzymes were assayed at a final concentration of 4 pmol/mL (for P450 1A1) or 10 pmol mL^{-1} (for P450 1A2 and 1B1) with the length of reaction time up to 40 min for P450 1A1 and 60 min for P450 1A2 and 1B1. To examine the linearity of the P450 1A1 reaction with time, 10 μM ClPyr was used as the substrate with 5 pmol mL^{-1} P450. For the S9 reactions, recombinant P450 was replaced by 1 mg mL^{-1} S9. Analyses of the Michaelis–Menten kinetic parameters and plots were performed by GraphPad Prism software version 6.0 for Windows (GraphPad Software, San Diego, CA).

2.5.3.3. LC analysis of ClPyr metabolites

LC-tandem mass spectrometry (LC-MS/MS) analyses were performed on a liquid chromatograph (ACQUITY UPLC system I-Class, Waters) coupled to a tandem quadrupole mass spectrometer (Xevo TQ-S, Waters). Chromatographic separation was performed on a C_{18} column (CORTECS C_{18}, 100 mm, 2.1 mm i.d., 1.6 μm, Waters). The column temperature was maintained at 40°C and gradient elution (methanol; 70% in water to 100% in 10 min) was at a flow rate of 0.2 mL min^{-1}.

MS/MS was performed in the electrospray negative ionization mode with a capillary voltage of 3000 V, a cone voltage of -30 V, and a collision energy of 25 eV. The selected reaction monitoring (SRM) mode was used to quantify the *m/z* 250.8 to 214.8 transition for ClPyr mono-hydroxylated metabolites. For further verification, the *m/z* 252.8 to 214.8 transition was used for 8-chloropyren-1-ol and 6-chloropyren-1-ol and *m/z* 250.8 to 186.8 was used for 3-chloropyren-1-ol.

LC-PDA-FL analyses were performed on a liquid chromatograph (Alliance e2695, Waters) coupled to a photodiode array detector (2998, Waters) and a fluorescence detector (2475 Multi λ, Waters). Chromatographic separation was performed on a C_{18} column (CORTECS C_{18}, 150 mm, 4.6 mm i.d., 2.7 μm, Waters). The column temperature was maintained at 40°C and gradient elution (methanol; 70% in water to 100% in 20 min) was performed at a flow rate of 1.0 mL min^{-1}. The wavelength for

PDA analyses was set at 200-400 nm. The excitation and emission wavelengths were 348 nm and 391 nm for mono-hydroxylated metabolites in the LC-FL analyses, respectively. To determine the enzymatic kinetic parameters, we quantified each ClPyr metabolite using the standard curves from 0.001 to 1 μM ($R^2 > 0.997$). The detection limits for each metabolite were 0.001 μM in LC-FL analyses.

2.5.3.4. Synthesis of ClPyr Metabolites

The mono-hydroxylated metabolites of ClPyr were synthesized by the chlorination of 1-hydroxypyrene using a modification of the method previously reported for the chlorination of PAH (Ohura et al., 2004b). 1-Hydroxypyrene (1 mmol) was dissolved in 4 mL of propylene carbonate, and an equimolar amount of *N*-chlorosuccinimide was added to this solution while stirring. The mixture was allowed to react at 100°C for 3 h with stirring and then the reaction product was recrystallized in methanol and water. The solid product was collected on filter paper and dissolved in ethyl acetate then evaporated to dryness and re-dissolved in methanol. The solution was further purified by fractionation using LC equipped with a C_{18} column (CORTECS C_{18}, 150 mm, 4.6 mm i.d., 2.7 μm, Waters) and RP-amide column (Ascentis, 150 mm, 4.6 mm i.d., 5 μm, Sigma-Aldrich). The representative chromatograms of LC fractionation were showed in Fig. 17. The fractionated ClPyr metabolites were used for NMR analysis, and assays for

P450 enzyme activity, and yeast reporter genes. The purities of the synthesized ClPyr metabolites were >98% (determined by LC-PDA peak area)

2.5.3.5. Urine sample treatment

A urine sample was collected from the author, living in Osaka, Japan. Informed consent was obtained from the donor. Urine sample was assayed as previously described (Chetiyanukornkul et al., 2006) with some modifications. Briefly, a 100-mL aliquot of the urine sample was adjusted to pH 5.0 with 0.1 M HCl, then buffered with 200 mL of 0.1 M acetate buffer (pH 5.0) and incubated for 2 h at 37°C with *β*-glucuronidase/arylsulfatase (13,600 and 750 units, respectively). Using a vacuum manifold, the reaction mixture was then loaded onto a Bond Elut Jr C_{18} cartridge (Agilent Technologies, CA, USA) that had been primed with 3 mL methanol and 3 mL water. The cartridge was sequentially washed with 10 mL water and 10 mL of 40% methanol in water. The cartridge was completely dried under vigorous air flow before connecting it to a Sep-Pak Silica Plus cartridge (Waters), which was conditioned with 10 mL n-hexane. After washing with 10 mL n-hexane, the trapped metabolites were eluted with 10 mL n-hexane/ethyl acetate (9:1, v/v) through the two cartridges. The eluate was evaporated and the residue was re-dissolved in methanol, and sonicated. The solution was then injected into the LC-MS/MS system for SRM analysis.

2.5.3.6. Nuclear Magnetic Resonance (NMR) Analysis

^{1}H-NMR, COSY, ^{13}C-HMBC, and HSQC spectra were obtained on a Bruker AVANCE700 spectrometer (Bruker Biospin Co., Karlsruhe, Germany) at 30°C, using solvent (CD_3OD) signals as an internal standard. ^{1}H-NMR and ^{13}C-NMR scans were operated at 700.334 MHz and 176.116 MHz, respectively, for all analyses.

2.5.3.7. Yeast reporter gene assay

The assay procedure was based on previously described methods (Nagayoshi et al., 2015). Briefly, yeast from a glycerol stock was grown overnight at 30°C with shaking, in synthetic medium consisting of 0.17% yeast nitrogen base (without amino acids and ammonium sulfate), 0.5% ammonium sulfate, 2% D-glucose, and 0.13% amino acids and nucleic acid bases. One microliter of various concentrations of test chemicals dissolved in DMSO, 5 μL of yeast overnight culture, and 200 μL of synthetic medium containing 2% galactose (instead of glucose) were mixed and incubated at 30°C for 18 h. In this step, AhR and Arnt were expressed under the control of the bi-directional *GAL1/GAL10* promoter. When a test chemical binds to the AhR/Arnt complex, the resulting ligand-receptor complex binds the ligand-binding domains of the reporter plasmid introduced into the same yeast cell and triggers expression of the reporter gene, β-galactosidase. After measuring the cell density spectrophotometrically at 595 nm

(OD_{595}), 10 µL of cell suspension and 90 µL of Z-buffer (60 mM Na_2HPO_4, 40 mM NaH_2PO_4, 1 mM $MgCl_2$, 10 mM KCl, 2 mM dithiothreitol, and 0.2% sarcosyl) containing 1 mg L^{-1} *o*-nitrophenyl-β-D-galactopyranoside were mixed and incubated for 1 h at 37°C. The amount of *o*-nitrophenol produced by β-galactosidase was measured spectrophotometrically at OD_{405}. Galactosidase activity (measured as lacZ units) was calculated by the following formula: absorbance at 405 nm/(absorbance at 595 nm × ml of cell suspension added × min of reaction time). Beta-naphthoflavone (β-NF) was used as a positive control. For calculation of the half-maximal effective concentration (EC_{50}) values and other parameters for regression analysis, data were plotted on single-logarithmic charts and fitted using a 3-parameter logistic method by GraphPad Prism. Each assay was conducted three times.

2.5.4. Results and discussion

2.5.4.1 Identification of the ClPyr metabolites

PAHs are metabolized to various products by xenobiotic (drug)-metabolizing enzymes. P450s play a key role in the initial step of oxidation of PAHs to phenols and oxides. PAHs are known to act as AhR ligands and induce AhR-mediated transcription of P450 *1A1*, *1A2*, and *1B1* genes (Tang et al., 1996; Beedanagari et al., 2010). On the basis of

these reports, we exposed ClPyr to recombinant P450 1A1, 1A2, and 1B1 enzymes and identified the resulting metabolites. We found three potential ClPyr metabolites in the P450 1A1 reaction mixture and labeled them M1, M2, and M3 according to the order of elution from the C_{18} column (Fig. 18). These three metabolites were also observed in reactions with P450 1A2 and 1B1 as well as with pooled human liver S9. These findings indicated that the production of these metabolites could occur in the human body. The LC-MS/MS analyses provided the information about the molecular ion m/z 251 for these metabolites, and product ion scan analyses showed that the fragmentation patterns of M1 and M2 were different from that of M3 (Fig. 19)). The mass fragmentation of M1 and M2 involved desorption of hydrochloric acid $[M\text{-}HCl]^-$ while desorption of an additional carbon monoxide, $[M\text{-}HCl\text{-}CO]^-$, was observed for M3. It was previously reported that the active reaction sites of pyrene were at the C-1, 3, 6, and 8 positions (Luthe et al., 2002). We therefore expected that these three compounds were 3-chloropyren-1-ol, 6-chloropyren-1-ol, and 8-chloropyren-1-ol. The main mass fragmentation of M1 and M2 involved desorption of hydrochloric acid $[M\text{-}HCl]^-$. An additional desorption of carbon monoxide, $[M\text{-}HCl\text{-}CO]^-$, was observed in M3. Since hydrochloric acid desorption is likely to destabilize the ring and induce carbon monoxide desorption, we inferred that the chlorine atom and hydroxyl group existed in

the same ring of the pyrene molecule and it as 3-chloropyren-1-ol. The loss of carbon monoxide following the desorption of hydrochloric acid was reported in the case of dichloro catechol, which also has chlorine and hydroxyl in the same benzene ring (Ziagova and Liakopoulou-Kyriakides, 2007). The loss of hydrochloric acid was also reported in LC-MS/MS analysis as being involved in the main fragmentation pathway of chlorophenol (Sarrión et al., 2003). To determine the precise structures of M1, M2, and M3, we synthesized these metabolites by chlorination of 1-hydroxypyrene, purified them using LC fractionation, and analyzed by NMR. The FL spectra of these synthesized compounds are shown in Fig. 20.

The ^{13}C-NMR data indicated that the carbon bound to the hydroxyl group was shifted to the high frequency range due to electron un-shielding. This carbon (C_a; Fig. 21) correlated with H_c, H_m, and H_b in the HMBC spectra for M1 and M2 (Fig. 22(d) & 23(d)). However, our findings indicate that in M3, C_a correlated only with H_m and H_b in the HMBC spectrum (Fig. 24(d)). Furthermore, in the ^{1}H-NMR, the H_b spectrum appeared as a singlet (Fig. 24(a)). These results indicate that the chlorine atom in M3 is at the C_c position in the pyrene skeleton and in agreement with the LC-MS/MS analysis, we identified M3 as 3-chloropyren-1-ol (3-ClPyr-1-ol). In the M1 COSY spectrum, H_m had a correlation with H_l (Fig. 22(c)), and from the H_l in HSQC, we determined

122.0244 ppm as C_l (Fig. 22(d)). C_l did not have any correlation in HMBC (Fig. 22(d)). The chlorine atom in M1 was therefore identified at the C_j position in the pyrene skeleton. These findings identified M1 as 8-chloropyren-1-ol (8-ClPyr-1-ol). M2 showed a correlation between C_l and H_j in the HMBC analysis (Fig. 23(d)). However, C_h of M2 did not show any correlation with proton in HSQC and C_f also showed the lack of correlation with protons in HMBC. These results indicated that the chlorine atom was at the C_h position in the pyrene skeleton of this compound. This identified M2 as 6-chloropyren-1-ol (6-ClPyr-1-ol). The chemical structures of the three identified compounds are shown in Fig. 25. The same positions of hydroxylation were also observed in the metabolism of 1-nitropyrene (Toriba et al., 2007). Finally, we confirmed that the metabolites generated *in vitro* corresponded to these synthesized compounds by matching their LC retention times, MS information, and PDA spectra.

2.5.4.2 Metabolism of ClPyr by P450 Enzymes

The amounts of metabolites produced were determined using the LC-FL system. We showed the amounts of metabolites generated in an enzyme amount and reaction time-dependent manner in Fig. 26. ClPyr was used as the substrate at a final concentration of 2.5 μM, and the reaction time was 2.5 min for P450 1A1 and 10 min for P450 1A2 and 1B1. Under these conditions, the formation of metabolites increased

linearly for up to 4 pmol/mL P450 protein for 1A1 and 20 pmol/mL P450 protein for both 1A2 and 1B1. The time-dependent increase in metabolite production was observed at 2.5 μM ClPyr and the length of time to the equilibrium condition was shortest in P450 1A1 among enzymes. The enzyme kinetic parameters determined using the Hill equation for the three metabolites of ClPyr by the three P450 enzymes are listed in Table 19 and the plots are shown in Fig. 27. P450 1A1 had the highest V_{max} value, followed by 1B1 and 1A2; this indicated that P450 1A1 had the greatest ability to metabolize ClPyr. This result was also observed for pyrene (Kim et al., 2004). The most abundant metabolite produced by each enzyme was 6-ClPyr-1-ol. Although all metabolites were created by each P450 preparation, the proportion of metabolites differed. In the presence of P450 1A1 and 1A2, the production rates for 8-ClPyr-1-ol and 6-ClPyr-1-ol were approximately equal. However, P450 1B1 produced more 6-ClPyr-1-ol and less of the other metabolites. Reaction with all the P450 enzymes consistently produced the same three metabolites but the three P450 enzymes varied in their efficacy to metabolize ClPyr. This result is consistent with differences in the structures of the P450 1A1, 1A2, and 1B1 enzymes in regions associated with substrate access/egress, and regio- and stereo-selective substrate binding. The structure of the P450 1A1 enzyme was reported to prefer polycyclic aromatic hydrocarbons and other

planar molecules, while 1A2 displayed a preference for aromatic amines and heterocyclic compounds (Zanger and Schwab, 2013; Walsh et al., 2013). Therefore, the planar structure of ClPyr may better fit the P450 1A1 active site. The formation of oxidative metabolites from ClPyr with P450 1B1 was faster than that with 1A2 in our study. Shimada et al. also reported that the oxidative metabolites formations of acenaphthene and acenaphthylene with P450 1B1 were more rapid than those with 1A2 (Shimada et al., 2015). The active site of the P450 1A1 enzyme has been reported to share a closer similarity to that of P450 1B1 than that of 1A2 (Walsh et al., 2013). It has also been reported that P450 1B1 is able to catalyze the activation of both polycyclic aromatic hydrocarbons and aryl amines (Shimada et al, 1996). These facts could be one reason for the higher efficacy of 1B1 than that of 1A2 in metabolizing the substrate. These results suggest that the expression levels of P450 genes could affect the proportion of the ClPyr metabolites produced in humans. The lowest level of product for all the enzyme types was for 3-ClPyr-1-ol. Hydroxylation by P450 is thought to be affected by steric hindrance of the chlorine atom. The enzyme reaction curve plot was well fitted by the Hill equation, which gave a smaller residual sum of squares than that of the Michaelis–Menten plot in each enzyme. These results indicate that the oxidation of ClPyr shows ligand cooperativity behavior in these P450 enzymes. In the case of

pyrene 1-hydroxylation in rabbit and human P450 1A2, it is reported that the plot of the oxidation rate showed a sigmoidal curve and fitted to a Hill expression (Sohl et al., 2008). In our study, h values in the Hill plot are from 1.4 to 2.4 and these values indicate that hydroxylation of ClPyr showed positive cooperativity. Sohl et al reported that the enzyme kinetic results are affected by substrate concentration, product inhibition, prolonged incubation time etc. in cases of weak cooperativity (Sohl et al., 2008). Hence, further study is needed to determine the reason for cooperativity behavior on ClPyr hydroxylation in P450 enzymes. Miranda et al. (2006) reported that the K_m and V_{max} values for zebrafish CYP 1A toward B[*a*]P were 4.17 μM and 0.94 nmol $min^{-1}nmol^{-1}$ P450, respectively. We calculated that the K_m and V_{max} values for 6-ClPyr-1-ol production by P4501A1 were 5.2 μM and 5.4 pmol min^{-1} $pmol^{-1}$ P450, respectively. Comparison of these results showed that these compounds had similar binding affinities for P450 1A1, but this enzyme metabolized ClPyr much more rapidly than B[*a*]P. This indicated that ClPyr is likely to be much less stable than B[*a*]P in the human body. We also detected these metabolites in a human urine sample analyzed by LC-MS/MS with SRM mode (Fig. 28). This showed that the ClPyr metabolites detected in this study are produced in human body and may therefore provide biomarkers for exposure to ClPyr and other ClPAHs.

2.5.4.3 ClPyr Metabolite AhR Ligand Activities

In this study, we used a yeast reporter gene assay to evaluate the AhR agonist activity of these metabolites. As shown in Fig. 29 and Table 20, all metabolites significantly activated the AhR, with similar EC_{50} values as the parent compound, ClPyr. The metabolite with the highest AhR ligand activity was 8-ClPyr-1-ol. 6-ClPyr-1-ol showed the lowest EC_{50} value, but the absolute intensity of its ligand activity was much lower than that of the other metabolites. The stability of compounds acting as AhR ligands relates closely to their toxicity. Continuous AhR activation induces a wide range of adverse biological effects, including mutagenesis, carcinogenesis, and immunological deficits (Duarte et al., 2013; Benson & Shepherd, 2011). Compounds which are considered non-toxic have also been shown to undergo rapid metabolism by AhR-induced phase I enzymes such as P450 1A1, limiting their ability to remain active as AhR ligands (Adachi et al., 2004).

However it has also been reported that the metabolites of 3-methylindole themselves have the ability to activate the AhR mechanism and induce the P450 1A1 enzyme (Weems & Yost 2010). Therefore, an examination of whether the ClPyr metabolites maintain AhR agonist activity represents an initial and important step towards evaluating the toxicity of ClPyr. The results of our study demonstrated that the

metabolites of ClPyr, like those of 3-methylindole, are as efficient ligands of the AhR as the parent compound.

Taken together, these findings indicated that once ClPyr is taken into the human body, it activates the AhR, induces AhR-related genes (such as P450s), and is subsequently metabolized by these enzymes. In addition, the resulting metabolites of ClPyr produced by phase I reactions retain equivalent AhR ligand activity.

2.5.5. Interim summary

In this study, the author identified the phase I reaction metabolites of ClPyr and revealed the kinetics of their production by relevant drug-metabolizing enzymes for the first time. In addition, the author found that ClPyr metabolites retained AhR ligand activity. These results will provide a basis for further research to develop methods for the evaluation of ClPAH exposure and *in vivo* kinetics, and to determine the resultant biological effects. We consider that further research is needed to investigate the biological activities of ClPAH metabolites because other compounds acting on the AhR-mediated xenobiotic metabolizing system (such as B[*a*]P) can form highly mutagenic and carcinogenic metabolites.

Table 19 The kinetic parameters in Hill plot[a] for ClPyr metabolism by P450

Enzyme	Metabolites	K_A (μM)*	V_{max}* (pmol/min/pmol P450)	h*	R^2	Residual sum of squares (Hill plot)	Residual sum of squares (Michaelis-Menten)
P450 1A1	8-chloropyren-1-ol	5.0±0.39	4.0±0.15	2.2±0.31	0.9727	2.0	4.9
	6-chloropyren-1-ol	5.2±0.46	5.4±0.23	2.0±0.28	0.9712	3.7	7.8
	3-chloropyren-1-ol	5.4±0.39	1.2±0.044	2.4±0.39	0.9738	0.17	0.48
P450 1A2	8-chloropyren-1-ol	4.6±0.41	0.15±0.0054	1.4±0.13	0.9848	0.0013	0.0022
	6-chloropyren-1-ol	4.3±0.37	0.16±0.0057	1.4±0.13	0.9843	0.0016	0.0028
	3-chloropyren-1-ol	8.9±1.4	0.0082±0.00064	1.6±0.24	0.9650	7.5×10^{-6}	1.0×10^{-5}
P450 1B1	8-chloropyren-1-ol	5.5±0.37	0.24±0.0077	1.8±0.18	0.9856	0.0034	0.0099
	6-chloropyren-1-ol	3.3±0.15	0.52±0.0097	2.0±0.14	0.9917	0.010	0.054
	3-chloropyren-1-ol	9.4±0.67	0.20±0.0078	1.8±0.16	0.9886	0.0015	0.0043

a; $V = V_{max} \times \text{Substrate}^h / (K_A{}^h + \text{Substrate}^h)$ K_A; the concentration which gives the half of the V_{max}.
h; Hill coefficient.
*; Each parameter was shown with the standard error of the mean.

Table 20 Regression analysis and intensities of AhR activation by ClPyr and its metabolites

Compound	a	b	R^2	EC_{50} (nM)
β-NF	1.3	5.4	0.9817	2.2
ClPyr	0.52	5.2	0.9928	460
8-ClPyr-1-ol	0.51	6.4	0.9904	380
6-ClPyr-1-ol	0.58	1.7	0.9692	220
3-ClPyr-1-ol	0.56	5.5	0.9844	630

OD_{405}/OD_{595} values were plotted on single-logarithmic charts and fitted using a 3-parameter logistic method using the following formula: LacZ unit = a + (b - a) / (1 + $10^{(\log EC_{50} - \text{concentration})}$); where a is the value at concentration 0, b is the LacZ unit value at infinity, and R^2 is the coefficient of determination of the regression curve.

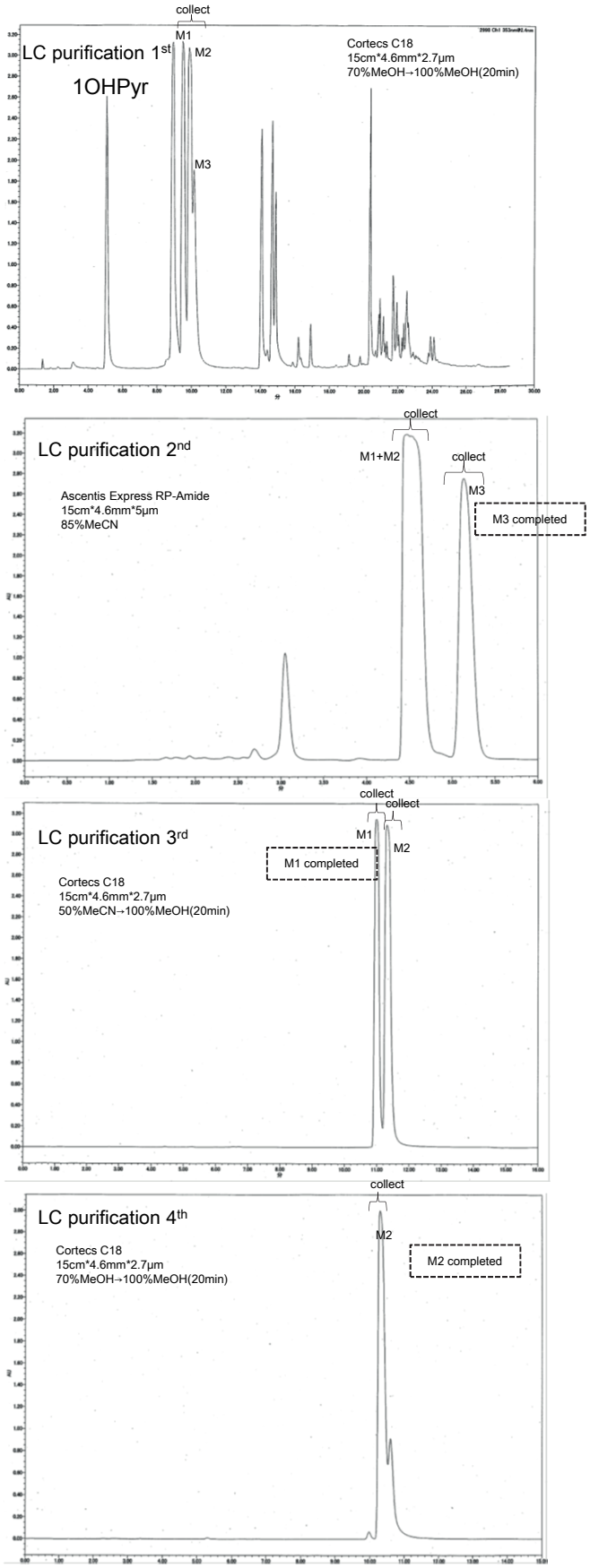


Fig.17 LC-PDA (353nm) chromatograms of synthesized mixture purification

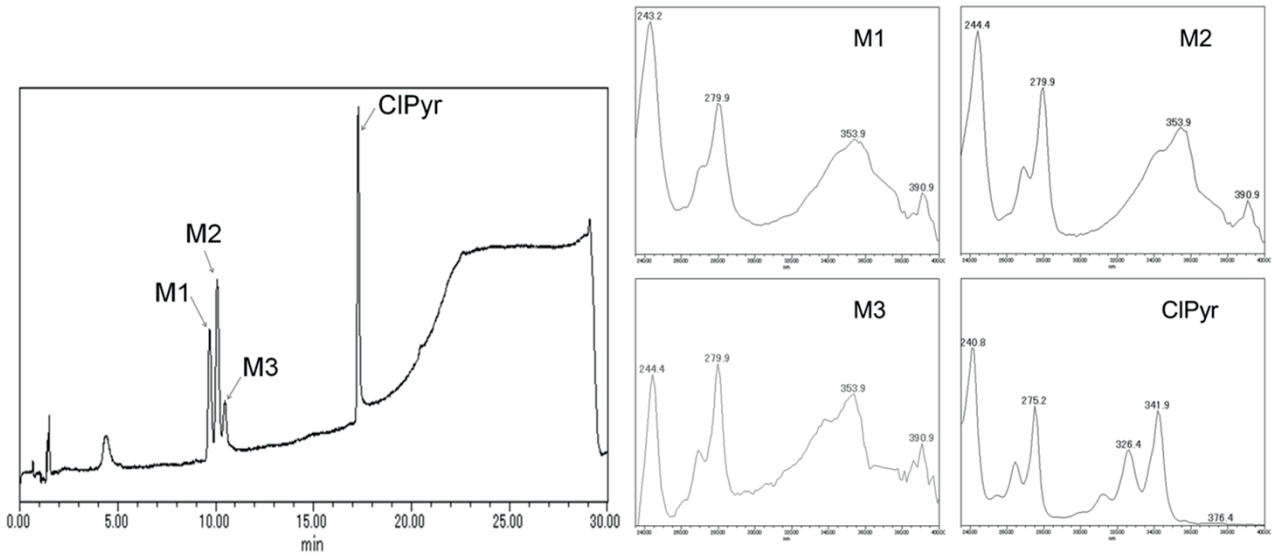


Fig.18 Chromatogram at 353 nm and PDA spectra (nm)
of the P450 1A1 reaction mixture

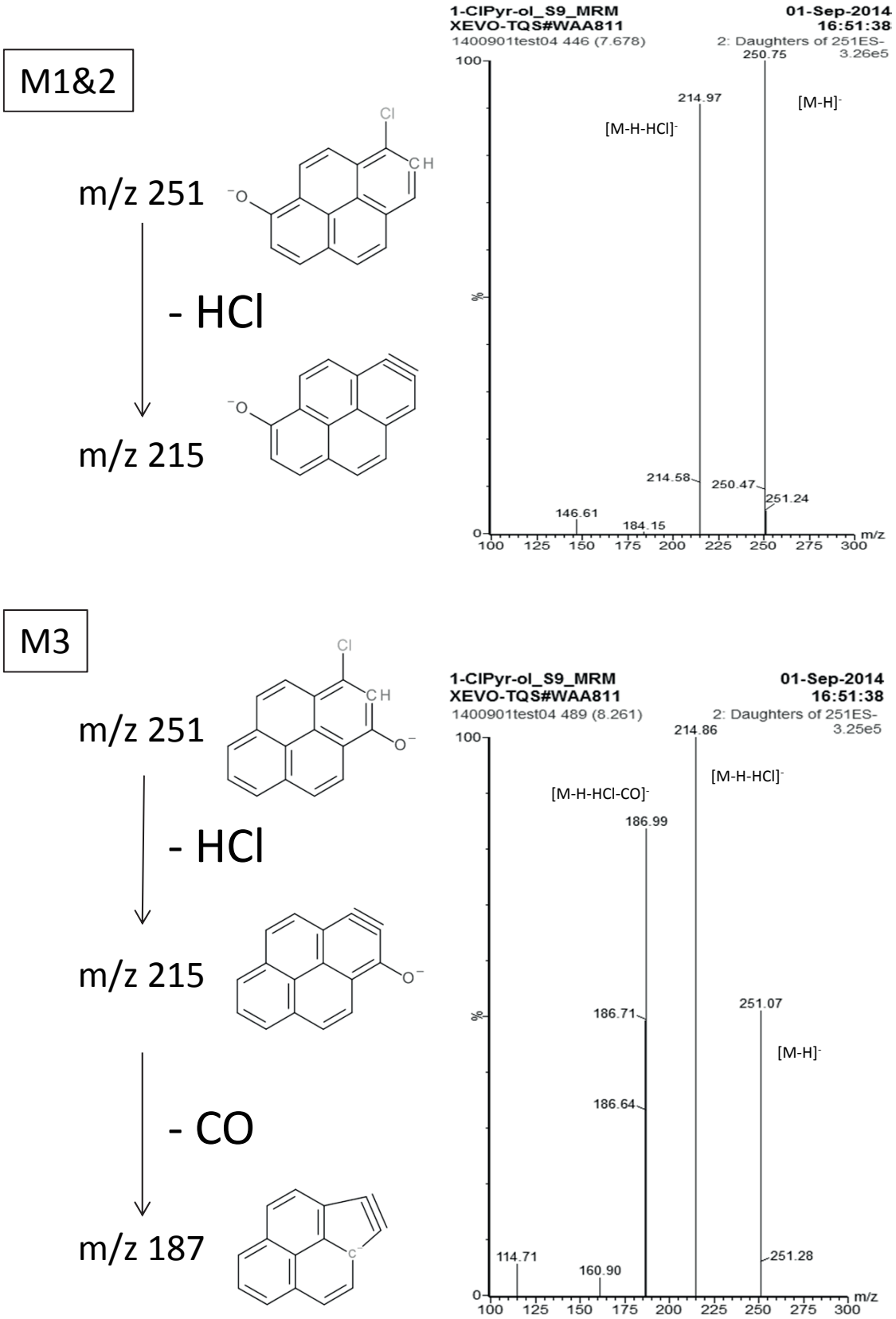


Fig.19 Expected fragmentation pathway of ClPyr metabolites in LC-MS/MS analyses

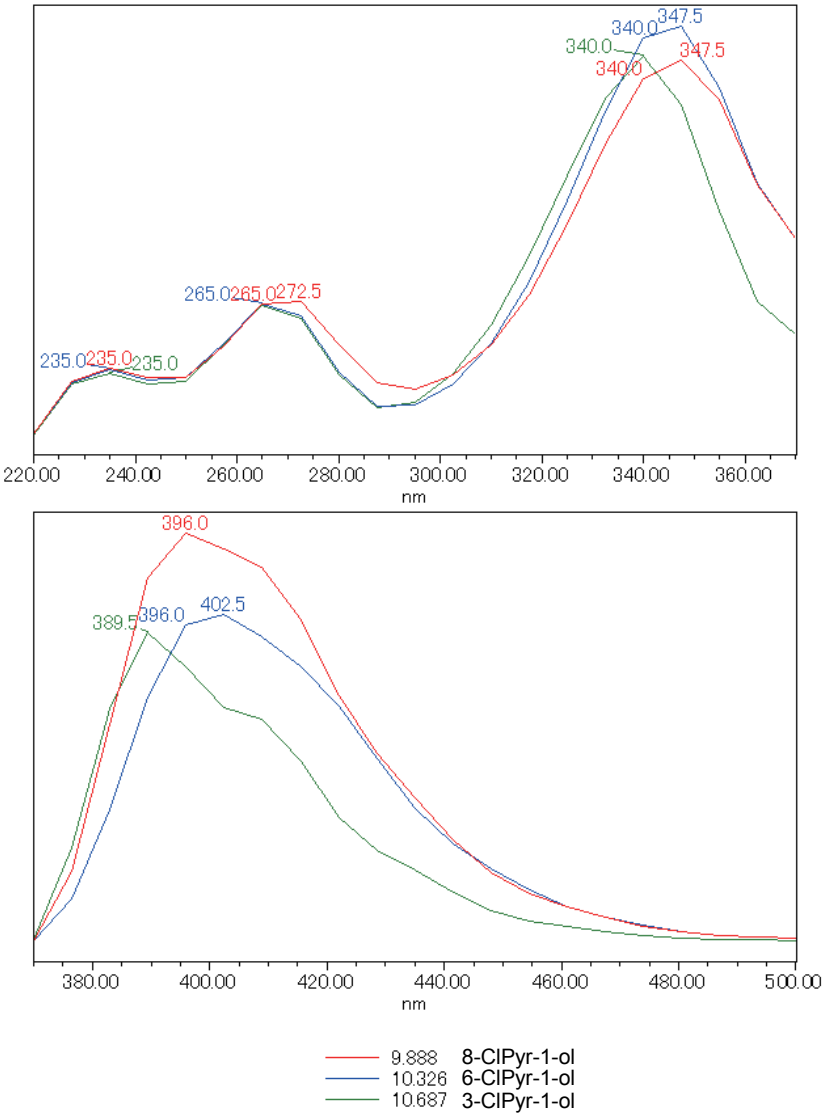


Fig.20 Excitation (λ_{em}=390 nm; upper) and emission(λ_{ex}=350 nm; lower) fluorescence scan spectra of ClPyr metabolites.

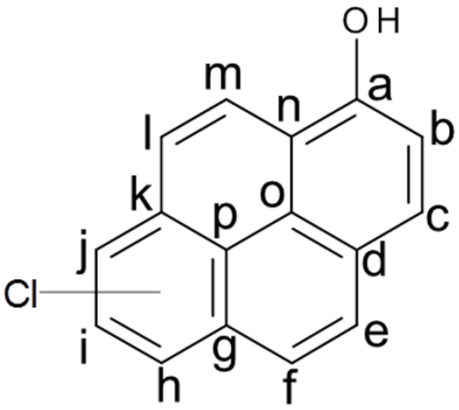


Fig.21 Letter code used for the NMR spectrum

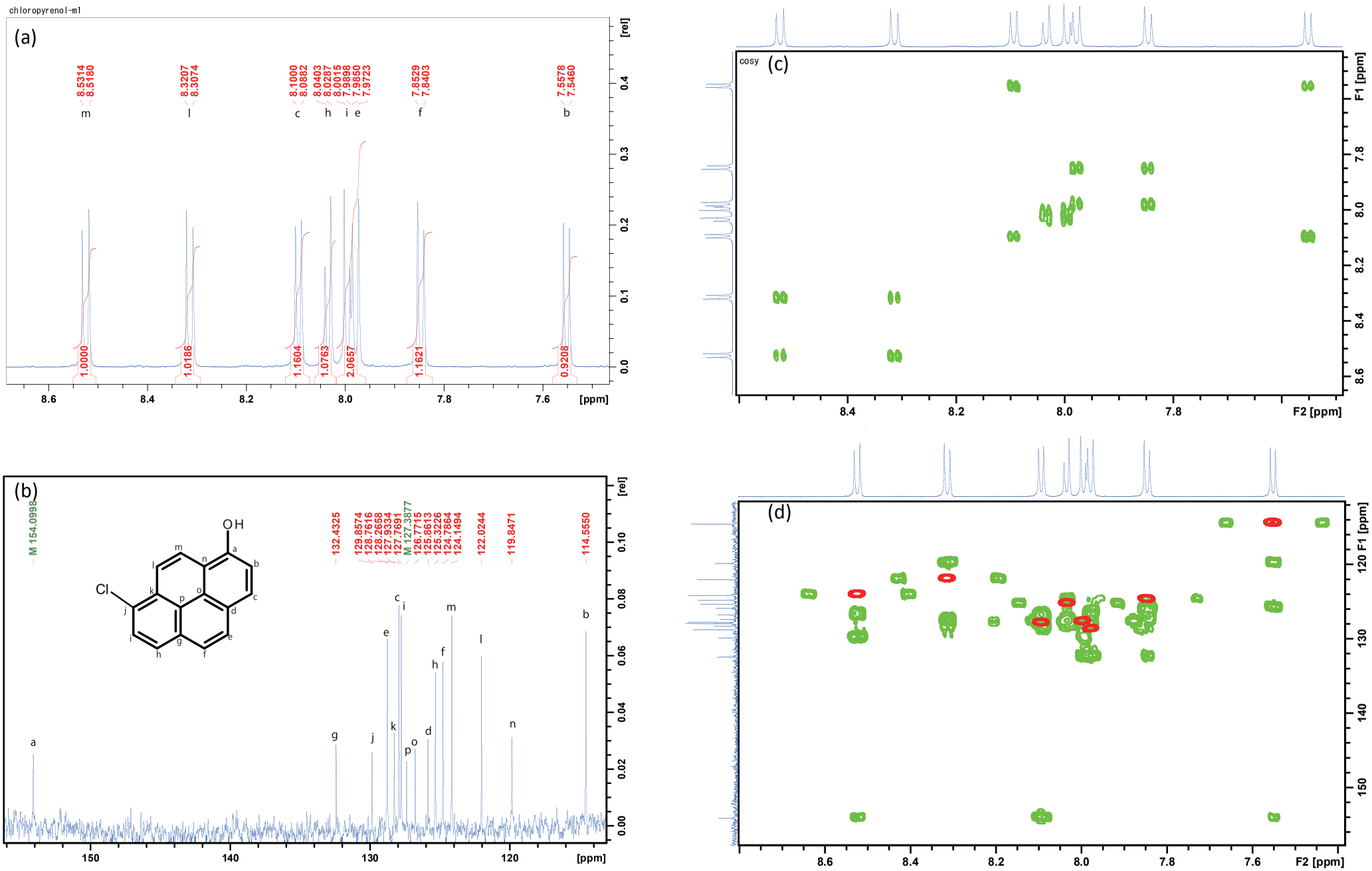


Fig. 22 (a) ^{1}H NMR spectrum of M1in CD_3OD (700 MHz), (b) ^{13}C NMR spectrum of M1in CD_3OD (176 MHz), (c) COSY spectra of M1 in CD_3OD, (d) HMBC(green)-HSQC(red) spectra of M1in CD_3OD

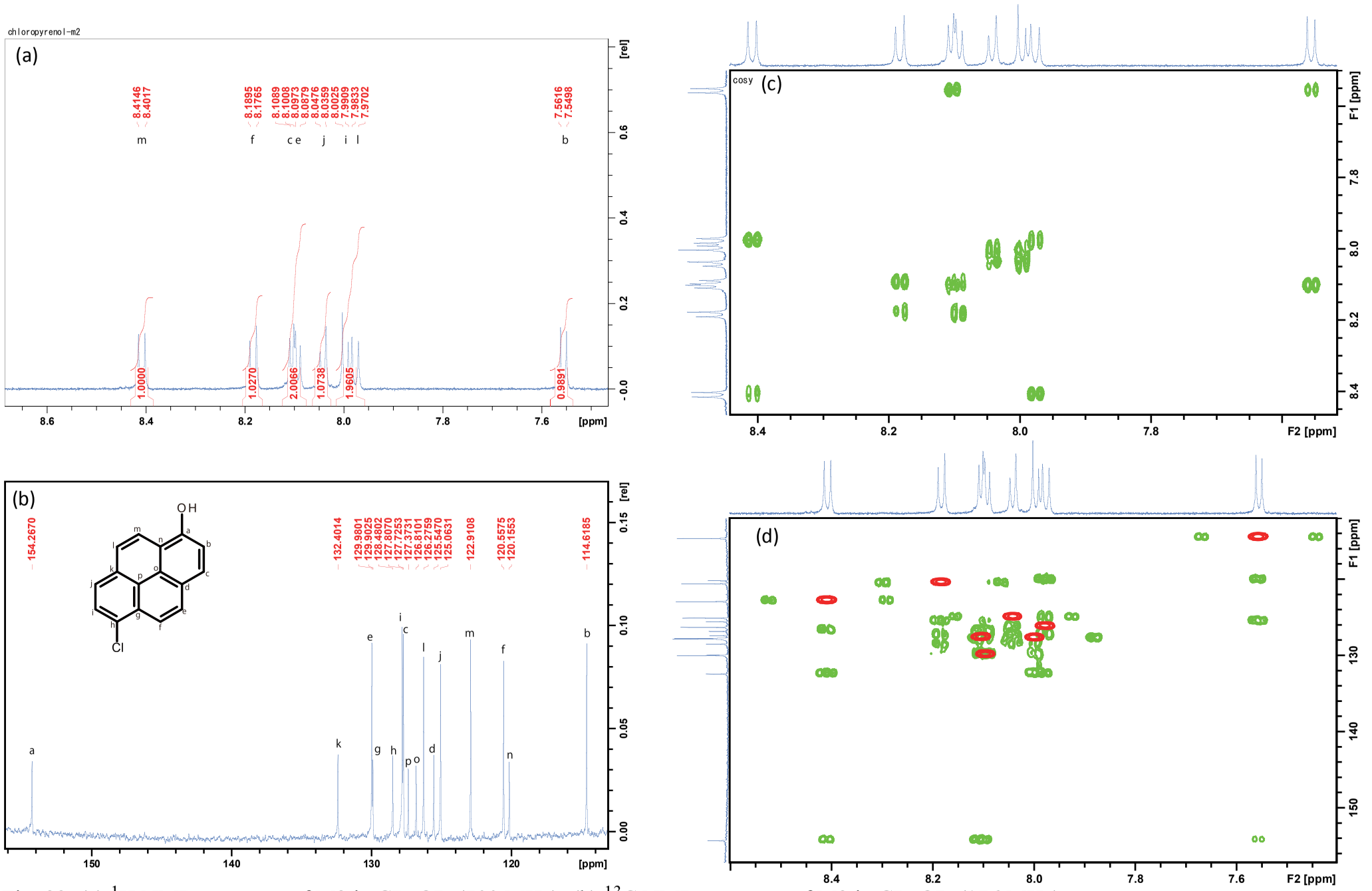


Fig. 23 (a) ^{1}H NMR spectrum of M2 in CD_3OD (700 MHz), (b) ^{13}C NMR spectrum of M2 in CD_3OD (176 MHz), (c) COSY spectra of M2 in CD_3OD, (d) HMBC(green)-HSQC(red) spectra of M2 in CD_3OD

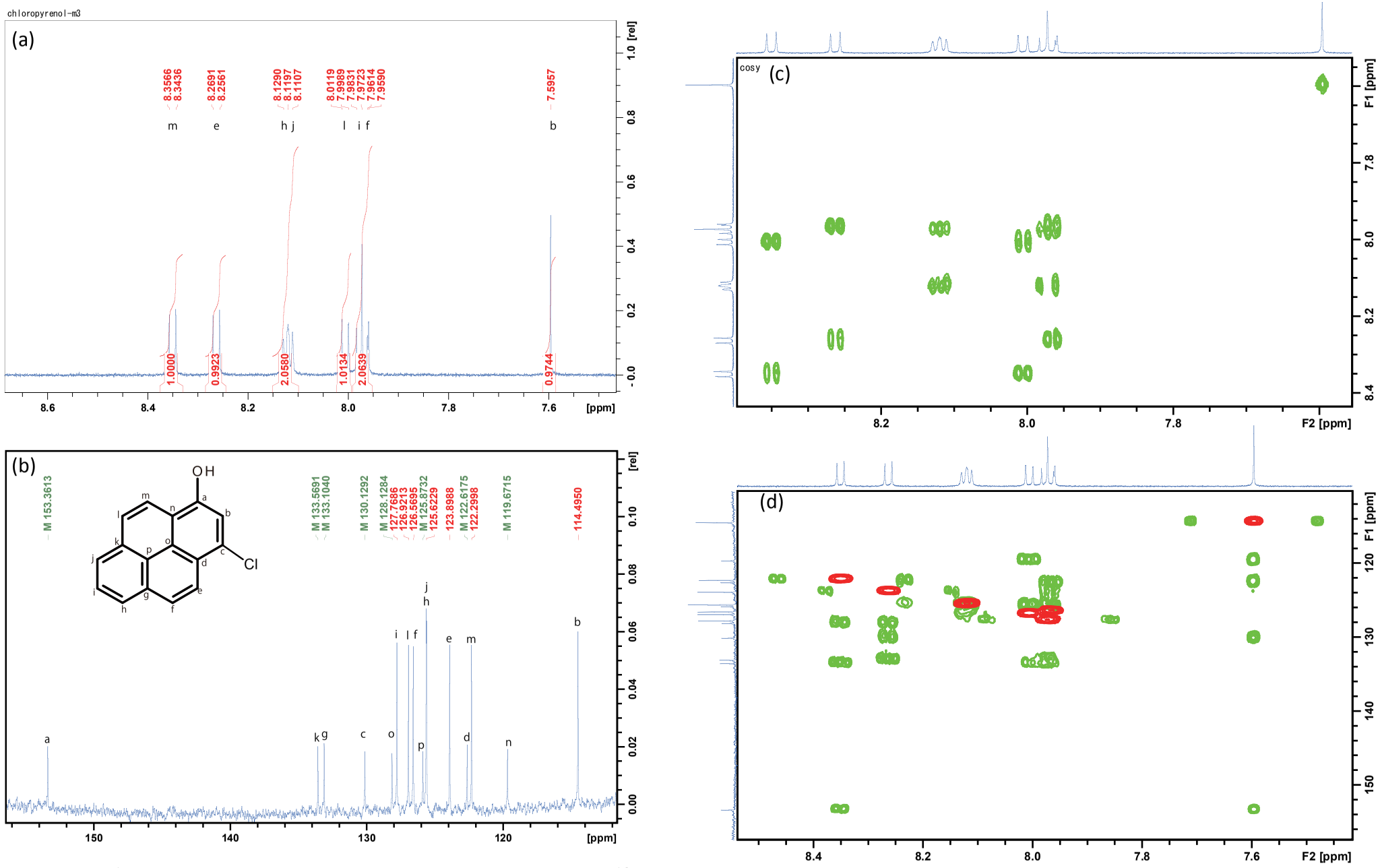


Fig. 24 (a) ^{1}H NMR spectrum of M3 in CD_3OD (700 MHz), (b) ^{13}C NMR spectrum of M3 in CD_3OD (176 MHz)
(c) COSY spectra of M3 in CD_3OD, (d) HMBC(green)-HSQC(red) spectra of M3 in CD_3OD

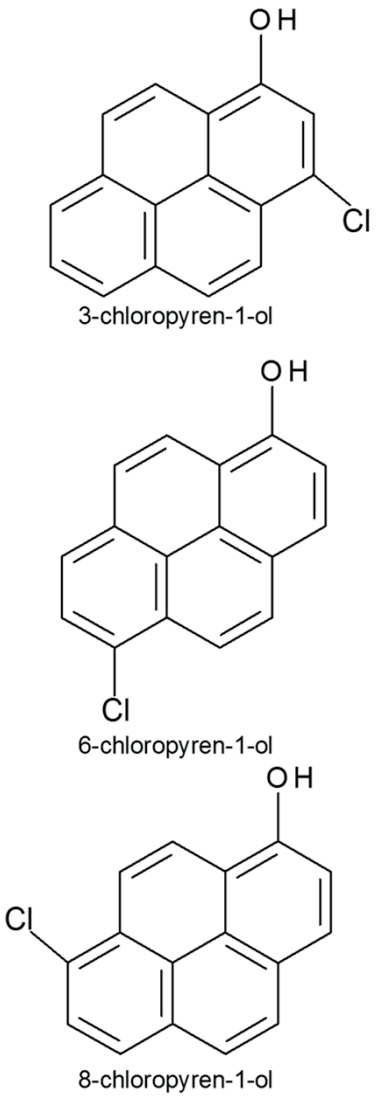


Fig.25 Chemical structures of mono-hydroxyl metabolites of ClPyr

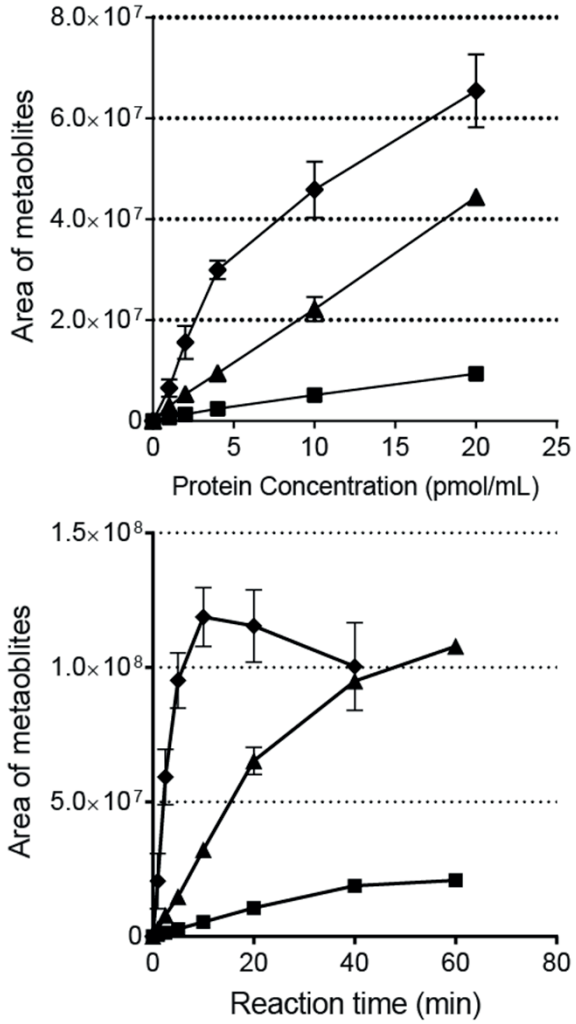


Fig.26 Formation of metabolites by P450 1A1, 1A2, and 1B1

Formation of metabolites by P450 1A1, 1A2, and 1B1. The upper panel shows the effect of the amount of enzyme on the production of metabolites (reaction time: 2.5 min for 1A1, 10 min for 1A2 and 1B1. ClPyr: 2.5 μM for each enzyme). The lower panel shows production of metabolites over time (amount of protein: 4 pmol /mL for 1A1, 10 pmol/mL for 1A2 and 1B1. ClPyr: 2.5 μM for each enzyme). Each plot indicates P450 1A1 (black diamond), P450 1A2 (black square), and P450 1B1 (black triangle). Error bars represent the standard deviation of triplicate samples.

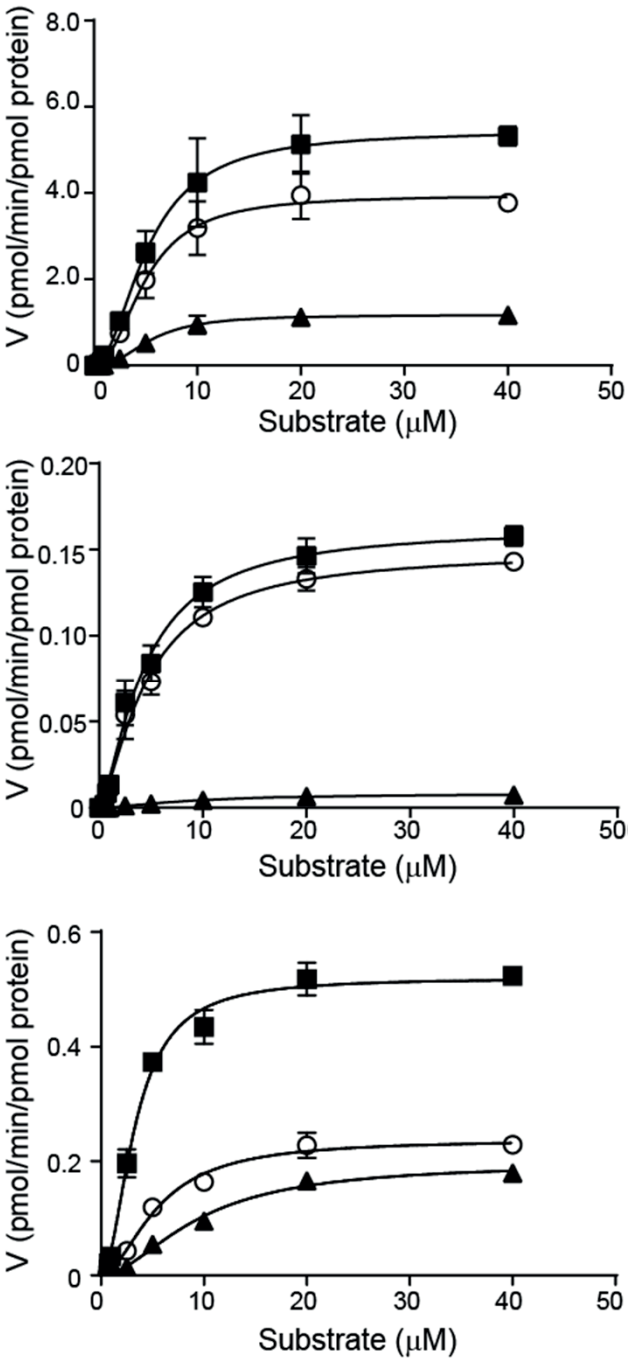


Fig.27 Hill equation plots of ClPyr metabolism by P450
The upper panel shows the plots of P450 1A1, the middle shows 1A2, and the lower shows 1B1 (reaction time; 40 min for 1A1, 60 min for 1A2 and 1B1. Amount of protein: 4 pmol /mL for 1A1, 10 pmol/mL for 1A2 and 1B1.). Nine concentrations (0, 0.5, 0.75, 1, 2.5, 5, 10, 20, and 40 μM) of ClPyr were assayed. Each plot indicates 8-ClPyr-1-ol (circle), 6-ClPyr-1-ol (black square), and 3-ClPyr-1-ol (black triangle). Error bars represent the standard deviation of triplicate values.

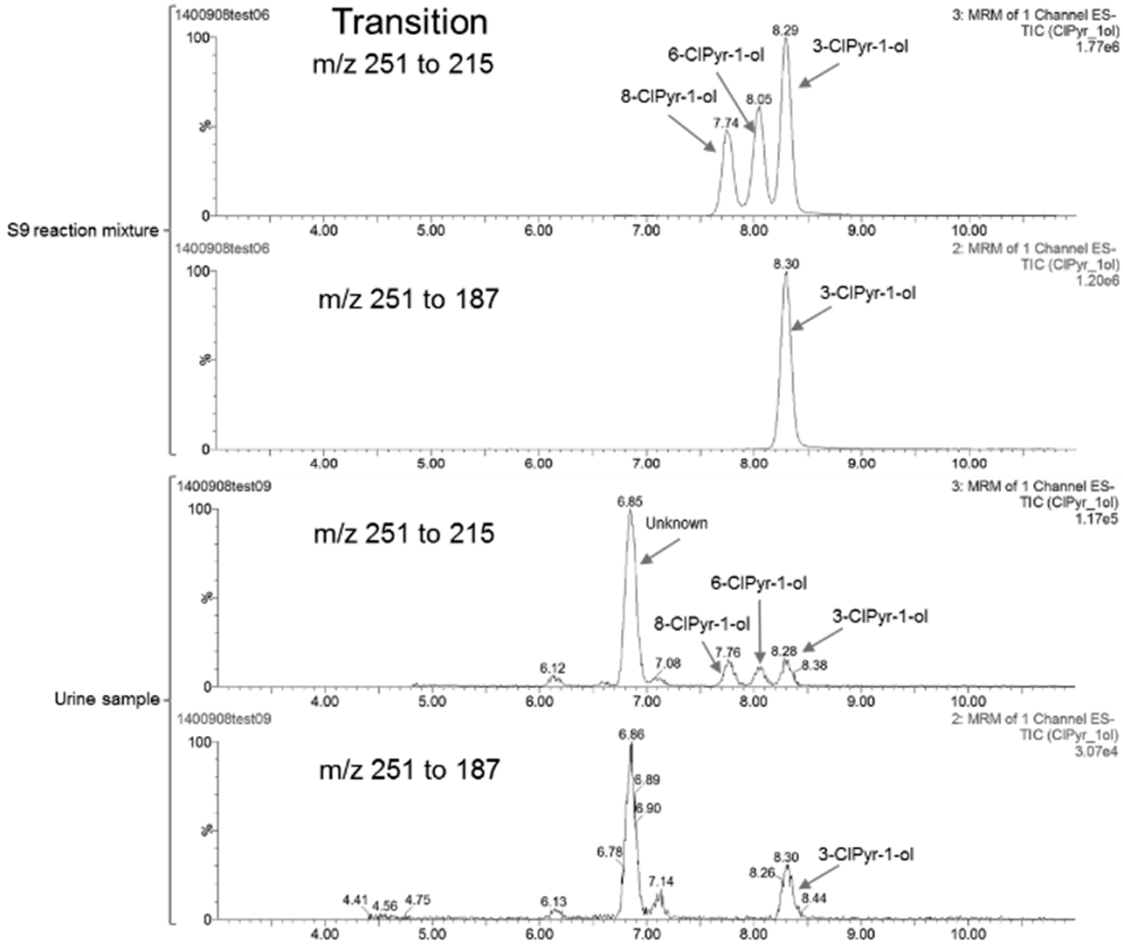


Fig.28 LC-MS/MS SRM chromatograms of S9 reaction mixture and human urine.

The top two chromatograms were obtained from human liver S9 reaction mixture and the bottom two were generated from analysis of urine.

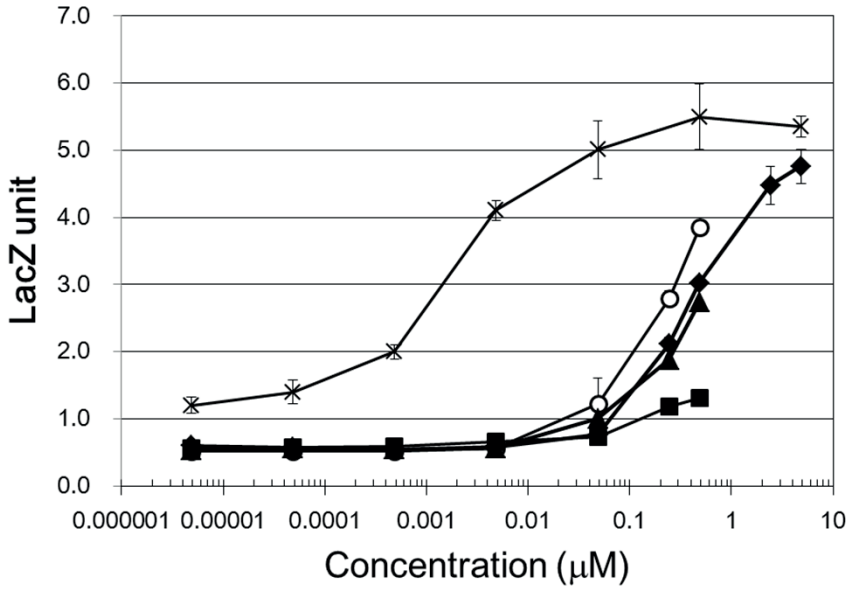


Fig.29 The AhR ligand activities of ClPyr and its metabolites
Plot shows Cl-Pyr (black diamond), 8-ClPyr-1-ol (circle), 6-ClPyr-1-ol (black square), 3-ClPyr-1-ol (black triangle), and β-NF (cross). β -NF was used as a positive control. Error bars show the standard deviation of each test.

2.6. Size distribution of chlorinated polycyclic aromatic hydrocarbons in atmospheric particles

2.6.1. Abstract

In this study, the particle size distributions of particulate matter (PM), polycyclic aromatic hydrocarbons (PAHs), and chlorinated polycyclic aromatic hydrocarbons (ClPAHs) were determined. The PM was collected using a PM0.1 air sampler, equipped with six stages of Teflon and inertial fiber filters. PM was collected in October 2014 and January 2015, allowing seasonal variations in the atmospheric behaviors of PM, PAHs, and ClPAHs to be identified in each particle size fraction. In addition, the aryl hydrocarbon receptor (AhR) ligand activity of extracts from each size fraction of the PM was evaluated. From this, it was found that the extracts of PM smaller than 2.5 μm show the strongest AhR ligand activity.

2.6.2. Introduction

In recent times, studies have revealed the negative effects of atmospheric particulate matter (PM) on human health. The International Agency for Research on Cancer (IARC) classified PM from outdoor air pollution into Group 1, meaning it is carcinogenic to humans, based on the evidence linking PM, and especially $PM_{2.5}$, to lung cancer risk (Krewski 2009, Loomis et al. 2013). It has also been reported that PM that reaches the lungs can directly and indirectly affect remote cardiovascular (CV) territories, and mediate CV disease (Brook 2008). It is known that the chemical compositions and sources of PM vary according to its particle size (Cheng et al. 2015), and almost all nanoparticles smaller than 100 nm are thought to reach and be deposited

within the lungs (Bolch et al. 2003). Therefore, considering the human health risk posed by PM in general, it is important to obtain a deeper understanding by determining the chemical compositions of PM of different size fractions. The main constituent of PM is organic carbon, in the form of carboxylic acids, alkanes, and polycyclic aromatic hydrocarbons (PAHs)(Li et al. 2013). Among the PAHs, a number of compounds, such as Benz[*a*]anthracene, Chrysene, and Benzo[*a*]pyrene, are classified as carcinogens by the IARC. In recent decades, certain PAH derivatives, known as chlorinated PAHs (ClPAHs), have attracted considerable interest as a new class of environmental contaminant. Studies have been conducted into ClPAHs in incineration ash (Horii et al. 2008), the atmosphere (Ohura et al. 2008; Kakimoto et al. 2014), and sediments (Ohura et al. 2015), and have partly revealed their generation mechanisms and behaviors in the environment. However, the particle size distribution of atmospheric ClPAHs is still unknown. Moreover, there are concerns that ClPAHs can have an adverse effect on human health, based on reports that some ClPAHs exhibit a mutagenic potential in *Salmonella* Typhimurium strains TA98 and TA100 (Bhatia et al. 1987, Colmsjö et al. 1984), and show stronger aryl hydrocarbon receptor (AhR)-mediated activities than those of the parent PAHs in yeast assays (Ohura et al. 2007). For this reason, atmospheric ClPAHs in fine particles that can easily reach the lung periphery are of

particular concern, and thus we consider it important to determine the concentrations of ClPAHs with respect to particle size, and to evaluate the effects of ClPAHs in atmospheric PM on human health. In this study, we determine the concentrations of PAHs and ClPAHs in atmospheric particles across six size fractions, collected and separated using a $PM_{0.1}$ air sampler (Furuuchi et al. 2010). Further, we evaluate the AhR ligand activity of silica-gel purified extracts from air particulates, in order to assess their potential risk to human health.

2.6.3. Methods

Samples were collected in a research laboratory in the eastern urban area of Osaka City, Japan, which is one of the largest cities in Japan. Air samples were collected using a $PM_{0.1}$ air sampler (Fig. 30) (Furuuchi et al. 2010) equipped with Teflon and inertial filters, with a flow rate of 40 L min^{-1}. Sampling took place on October 6, 7, 8, and 9 in 2014, and January 13, 16, 19, and 22 in 2015. The filters were replaced every 24 hours. After sampling, the filters were packaged with aluminum foil and stored in a freezer until needed for analysis. The target of ClPAHs and PAHs analyzed in this study are 1-ClPy, 6-ClBaP, Phe, Ant, Pyr, Chry, BaA, BbF, BkF, BaP, and Pery and information of chemicals are described earlier in section 2.4.3.2. The treatments for the atmospheric

particle samples are described in 2.4.3.3. The GC/MS analysis conditions are described in 2.4.3.4. The experimental conditions for the yeast reporter gene assay are described in 2.5.3.7. and a slightly modified preparation method for PAHs and ClPAHs analyses was employed in this study. Specifically, the extraction was performed without the addition of an internal standard. Additionally, 20 μL of dimethyl sulfoxide (DMSO) was added to the eluent after silica gel purification, and evaporated until the hexane and dichloromethane were completely removed, before use in the yeast reporter gene assay. Half of the filter samples that were collected in January 16 were used for the yeast reporter gene assay.

2.6.4. Results and discussion

As illustrated in Fig. 31, the most abundant PM particle size fraction in October was 10 –2.5 μm. However, in January, the PM within the 2.5–1.0 μm, 1.0–0.5 μm, and 0.5–0.1 μm size fractions increase markedly, and the highest abundance was shifted to 1.0–0.5 μm. Therefore, although approximately 50% of PM exists as particles larger than 2.5 μm in October, in January, the PM belonging to these larger fractions decreases to less than 30%.

Among the ClPAHs, 1-ClPyr and 6-ClBaP were detected in 44 out of 48 samples and

37 out of 48 samples, respectively. Target PAHs were detected in all 48 filters, with the exception of Phenanthrene in PM samples >10 μm in October. In both October and January, the maximum concentrations of ClPAHs and PAHs were found in particles ranging from 1.0 to 0.5 μm in size (Fig. 32).

The distributions of ClPAH and PAH concentrations (pg m^{-3}) among the different particle size fractions are shown in Fig. 33. This figure reveals that, in both sampling seasons, over 80% of PAHs and ClPAHs exist in the particles that are smaller than 2.5 μm, which are expressly understood to adversely affect human health. PAHs and ClPAHs have roughly the same distribution patterns in both sampling periods, but as with overall PM distributions, the concentrations of PAHs and ClPAHs markedly increase in the 2.5–1.0 μm and 1.0–0.5 μm fractions in January. PAH and ClPAH concentrations in the nanoparticle fraction (<0.1 μm) were between 11 and 26% of the total concentrations in October, and between 6 and 14% in January. These results demonstrate that PAHs and ClPAHs are present in the nanoparticle phase, which can easily reach and be deposited in the lung peripheries.

Particulate matter is generally classified into three modes, according to particle size. Among these, the 0.1–1.0 μm size fraction belongs to the ‘accumulation’ mode, whose particles have relatively long lifetimes in the atmosphere, compared with the other two

aerosol modes: ‘nuclei’ and ‘coarse particles’ (Hinds 1999). Therefore, it is possible that the observed increases in PM, PAHs, and ClPAHs in the 1.0–0.5 μm size fraction in January are the result of remote contamination sources. Back trajectory analyses for all sampling dates show that the airmasses during January were derived from the Asian continent, however no particular tendencies were identified in October (Fig. 34) (Stein et al. 2015). This suggests that the airmasses from the Asian continent are a possible carrier of accumulation mode PM, along with PAHs and ClPAHs. Further, it is possible that the ClPAHs were generated from PAHs within the PM during atmospheric transportation. Because it is reported that natural photochemical reactions of PAHs are responsible for the occurrence of ClPAHs (Sankoda et al. 2013).

It is well known that PM has carcinogenic characteristics, but the clear causes of such risks are still unknown, due to its inherently complex chemical composition. Aryl hydrocarbon receptor (AhR) activity is one important indicator that can be used to evaluate carcinogenesis. Therefore, in order to investigate the relationship between particle size distributions and carcinogenesis, we evaluated the AhR ligand activity of each PM fraction from 16 January, 2015. Results of the yeast reporter gene assay are shown in Fig. 35. These analyses indicate that every particle size fraction contains AhR ligands, and that the extracts of PM smaller than 2.5 μm show the strongest AhR ligand

activity. The four PM size fractions smaller than 2.5 μm showed approximately equal AhR ligand activities. This result revealed that the $PM_{2.5}$ fraction is important for determining the effects of PM on cancers. The toxic equivalent concentrations (TEQs) for mixtures of ClPAHs and PAHs in the air particulates have been calculated, using the same equation as in our previous report (Kakimoto et al. 2014), using the PAHs and ClPAHs concentrations in PM sampling on 16 January 2015, and the degree of toxicity compared to BaP, as determined by Ohura et al. (2007; 2009) for AhR-mediated activities of ClPAHs in a yeast assay. The calculated TEQs for Σ2ClPAH and Σ7PAH concentrations in the air particulates were high in the 2.5–1.0 μm and 1.0–0.5 μm fractions. This suggests that the atmospheric ClPAHs and PAHs may contribute to the AhR-induced toxic effects of airborne PM, especially for particles between 2.5 and 0.5 μm in size. Additionaly, the results also indicate that potential AhR ligands, other than PAHs and ClPAHs, exist within the 0.5– 0.1 and <0.1 μm size fractions.

2.6.5. Interim summary

In this study, the particle size distribution of ClPAHs has been revealed for the first time, demonstrating that 10–20% of ClPAHs exist in the nanoparticle phase, which can easily reach and be deposited in the lung periphery. It appears that airmasses derived from the Asian continent contribute to an increase in accumulation mode ClPAHs and

PAHs, as well as PM. In addition, this study has revealed that every particle size fraction contains AhR ligands, and that the extracts from PM smaller than 2.5 μm show the strongest AhR ligand activity.

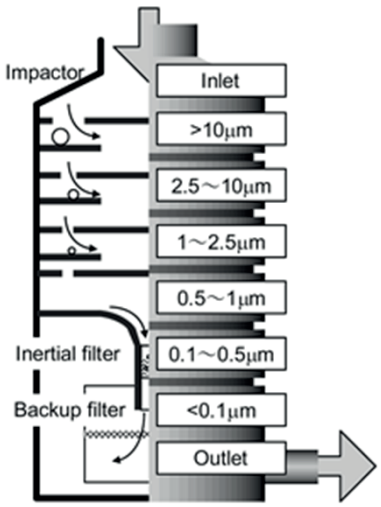


Fig.30 The shape of $PM_{0.1}$ air sampler

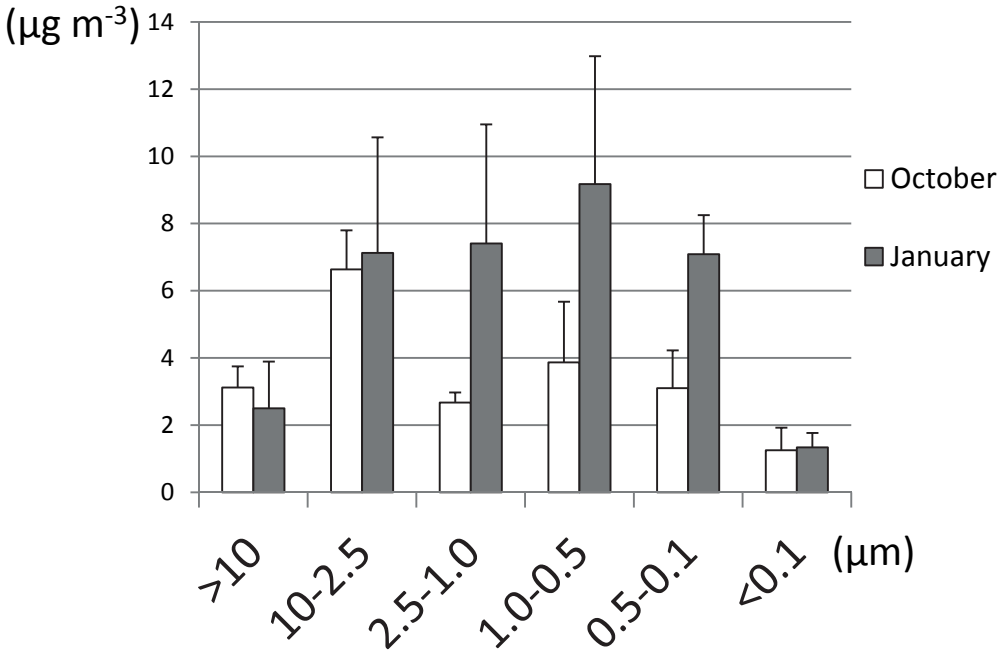


Fig.31 The concentration of PM in each size fraction

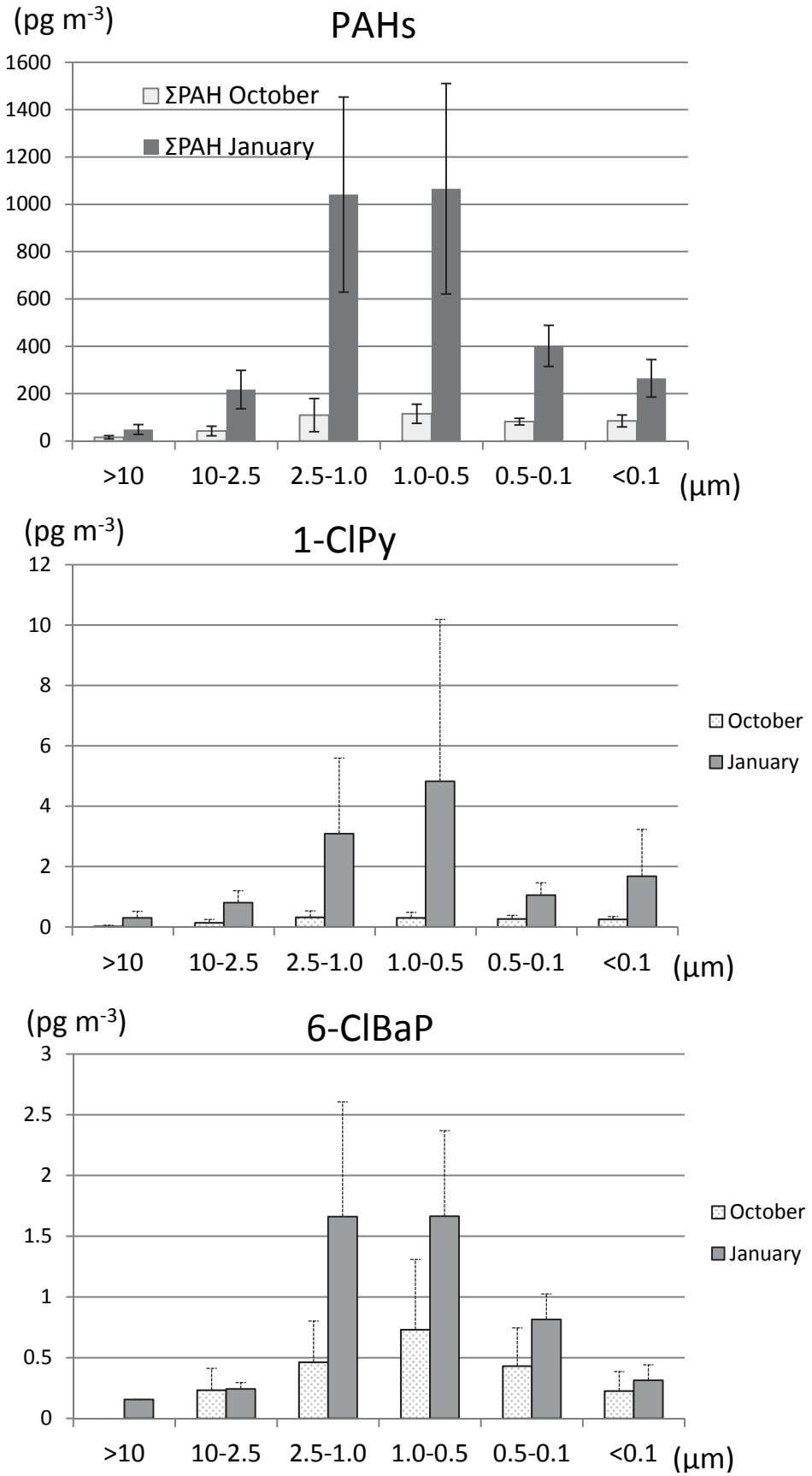


Fig.32 The concentration of PAHs, 1-ClPy and 6-ClBaP in each size fraction

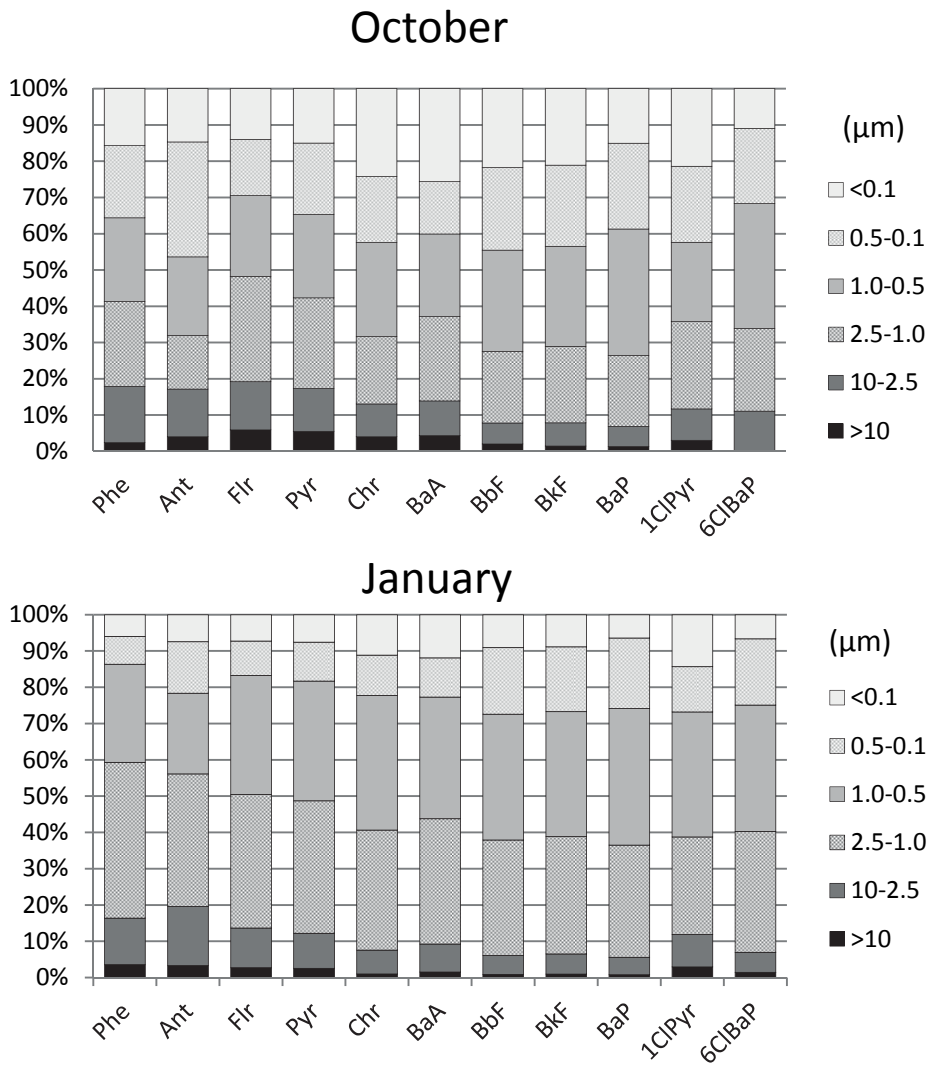


Fig.33 The size particle distributions of PAHs and ClPAHs

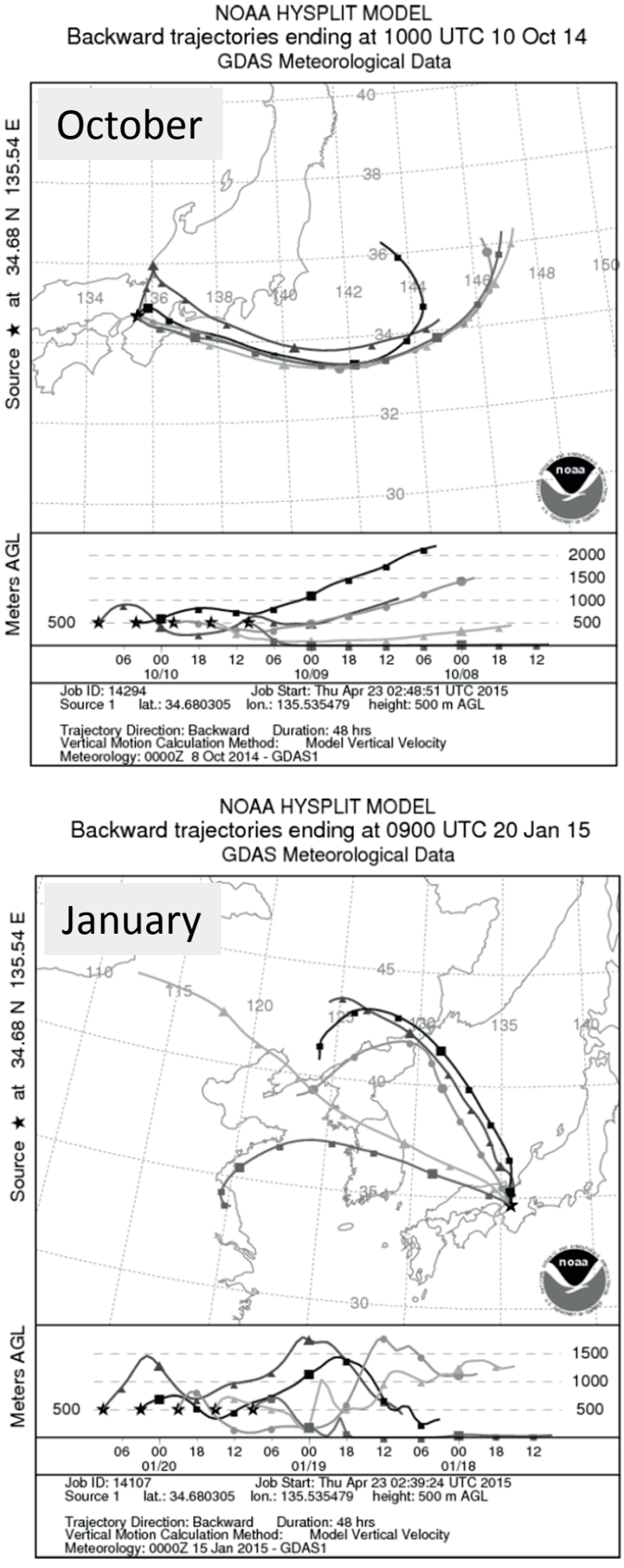


Fig.34 Representative results of back trajectory analysis over the sampling period. (upper, 10 Oct, 2014; lower, 20 Jan 2015)

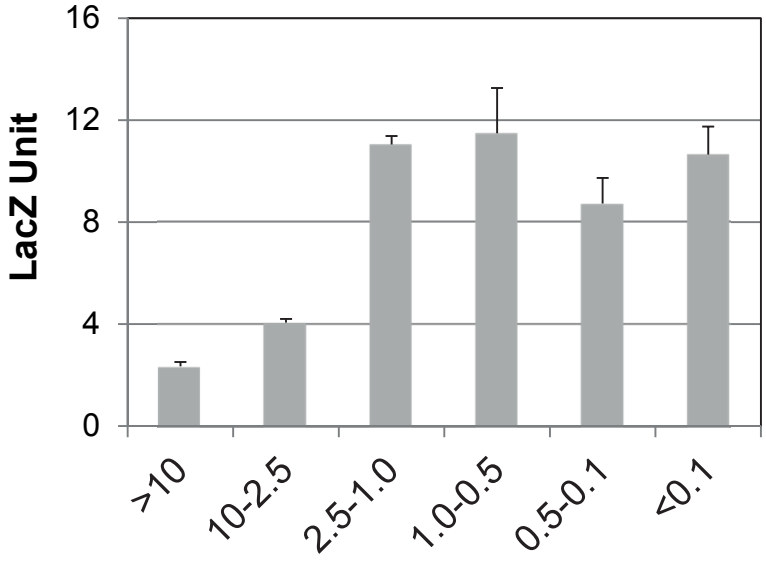


Fig.35 The AhR ligand activities of the extracts of each PM fraction

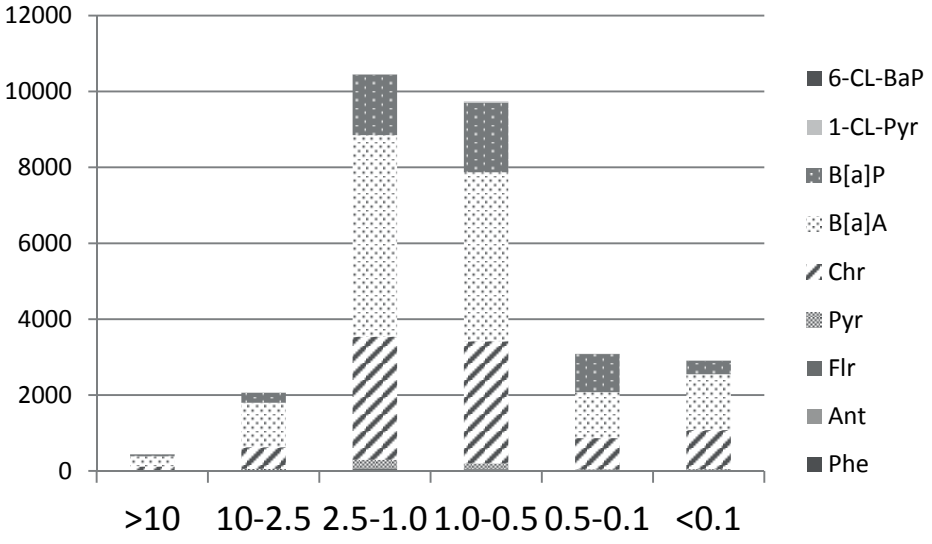


Fig.36 Toxic equivalent concentrations (TEQs) of ClPAHs and PAHs (fg-TEQ m^{-3}) in each PM fraction

3. Summary

This thesis aimed to reveal the pollution and exposure level of halogenated organic pollutants, such as Dechlorane Plus and chlorinated polycyclic aromatic hydrocarbons, which are not yet restricted, and we do not have enough information about their exposure levels in Japan.

In this thesis, the author have described the following about my research study and original discoveries and conclusions.

1. The DP concentrations in fish samples and revealed the correlation between halogenated flame-retardants (DP, PBDE, and HBCD). (*Section 2.1.*)
2. The dietary and inhalational DP exposure level in Japan and compared with legacy POPs (PBDEs). Their concentrations are not likely to cause adverse health effects in the general Japanese population. (*Section 2.2.*)
3. The atmospheric DP levels in East-Asian countries and DP particle size fraction distribution behavior. The results indicate that atmospheric DP contamination is from domestic sources, such as electrical wire, cable coatings, computer connectors, and plastic roofing materials, and in Japan is closely linked to urban centers with dense populations. (*Section 2.3.*)
4. The atmospheric ClPAH levels in East Asian countries and considered the effect of

the locational and seasonal variations of sampling on their behavior. The author estimated the toxic effects of atmospheric ClPAHs on each sampling city. (*Section 2.4.*)

5. The oxidative metabolites of ClPyr and revealed the kinetics of their production by relevant drug-metabolizing enzymes. In addition, the author found that ClPyr metabolites retained AhR ligand activity. (*Section 2.5.*)
6. The particle size distribution of ClPAHs was revealed for the first time and 10 to 20% ClPAHs exist in the nanoparticle phase which easily reach and deposit to lung periphery. In addition, the author found that the extracts of PM smaller than 2.5 μm show the strong AhR ligands activity. (*Section 2.6.*)

4. References

Adachi, J., Mori, Y., Matsui, S., Matsuda, T., 2004. Comparison of gene expression patterns between 2,3,7,8-tetrachlorodibenzo-p-dioxin and anatural arylhydrocarbon receptor ligand, indirubin. Toxicol Sci 80 (1), 161−169. DOI: 10.1093/toxsci/kfh129

Akutsu, K., Obana, H., Okihashi, M., Kitagawa, M., Nakazawa, H., Matsuki, Y., Makino, T., Oda, H., Hori, S., 2001. GC/MS analysis of polybrominated diphenyl ethers in fish collected from the Inland Sea of Seto, Japan. Chemosphere 44, 1325−1333. DOI: 10.1016/S0045-6535(00)00534-8

Akutsu, K., Kitagawa, M., Nakazawa, H., Makino, T., Iwazaki, K., Oda, H., Hori, S., 2003. Time-trend (1973-2000) of polybrominated diphenyl ethers in Japanese mother's milk. Chemosphere 53, 645−654. DOI: 10.1016/S0045-6535(03)00764-1

Baek, S.-Y., Jurng, J., Chang, Y.-S., 2013. Spatial distribution of polychlorinated biphenyls, organochlorine pesticides, and dechlorane plus in Northeast Asia. Atmos Environ 64, 40−46. DOI:10.1016/j.atmosenv.2012.09.015

Bakker, M.I., de Winter-Sorkina, R., de Mul, A., Boon, P.E., van Donkersgoed, G., van Klaveren, J.D., Baumann, B.A., Hijman, W.C., van Leeuwen, S.P., de Boer, J., Zeilmaker, M.J., 2008. Dietary intake and risk evaluation of polybrominated diphenyl ethers in The Netherlands. Mol Nutr Food Res 52, 204−216. DOI: 10.1002/mnfr.200700112

Beedanagari, S.R., Taylor, R.T., Bui, P., Wang, F., Nickerson, D.W., Hankinson, O., 2010. Role of epigenetic mechanisms in differential regulation of the dioxin-inducible human CYP1A1 and CYP1B1 genes. Mol. Pharmacol. 78, 608−616. DOI: 10.1124/mol.110.064899

Benson, J. M., Shepherd, D. M., 2011. Dietary ligands of the aryl hydrocarbon receptor induce anti-inflammatory and immunoregulatory effects on murine dendritic cells. Toxicol Sci 124 (2), 327−338. DOI: 10.1093/toxsci/kfr249

Bhatia, A.L., Tausch, H., Stehlik, G., 1987. Mutagenicity of chlorinated polycyclic aromatic compounds. Ecotox Environ Safe 14, 48-55. DOI: 10.1016/0147-6513(87)90082-0

Bi, X., Thomas, G.O., Jones, K.C., Qu, W., Sheng, G., Martin, F.L., Fu, J., 2007. Exposure of electronics dismantling workers to polybrominated diphenyl ethers, polychlorinated biphenyls, and organochlorine pesticides in South China. Environ Sci Technol 41, 5647−5653. DOI: 10.1021/es070346a

Bidleman, T., 1988. Atmospheric processes—Wet and dry deposition of organic compounds are controlled by their vapor-particle partitioning. Environ Sci Technol 22, 361−367. DOI: 10.1021/es00169a002

Bolch, W. E., Huston, T. E., Farfán, E. B., Vernetson, W. G., Bolch, W. E., 2003. Influences of parameter uncertainties within the ICRP-66 respiratory tract model: particle clearance. Health Phys 84, 421-435.

Brock, W.J., Schroeder, R.E., McKnight, C.A., VanSteenhouse, J.L., Nyberg, J.M., 2010. Oral repeat dose and reproductive toxicity of the chlorinated flame retardant Dechlorane Plus. Int J Toxicol 29, 582–593. DOI: 10.1177/1091581810384154

Brook, R. D., 2008. Cardiovascular effects of air pollution. Clin Sci (Lond) 115, 175-87. DOI: 10.1042/CS20070444

Buchet, J.P., Gennart, J.P., Mercado-Calderon, F., Delavignette, J.P., Cupers, L., Lauwerys, R., 1992. Evaluation of exposure to polycyclic aromatic hydrocarbons in a coke production and a graphite electrode manufacturing plant: assessment of urinary excretion of 1-hydroxypyrene as a biological indicator of exposure. Br J Ind Med 49, 761–768.

Chen, M.L., Wang, L., Chi, Y.K., Mao, C.C., Lung, S.C., Mao, I.F., 2012. Polybrominated diphenyl ethers in foodstuffs from Taiwan: level and human dietary exposure assessment. Sci Total Environ 431, 183–187. DOI: 10.1016/j.scitotenv.2012.05.046

Chen, S.J., Tian, M., Wang, J., Shi, T., Luo, Y., Luo, X.J., Mai, B.X., 2011. Dechlorane Plus (DP) in air and plants at an electronic waste (e-waste) site in South China. Environ Pollut 159, 1290–1296. DOI: 10.1016/j.envpol.2011.01.026

Cheng, Y., Lee, S., Gu, Z., Ho, K., Zhang, Y., Huang, Y., Chow, J. C., Watson, J. G., Cao, J., Zhang, R., 2015. PM2.5 and PM10-2.5 chemical composition and source apportionment near a Hong Kong roadway. Particuology 18, 96-104. DOI: 10.1016/j.partic.2013.10.003

Chetiyanukornkul, T., Toriba, A., Kizu, R., Makino, T., Nakazawa, H., Hayakawa, K., 2002. Determination of 1-hydroxypyrene in human urine by high-performance liquid chromatography with fluorescence detection using a deuterated internal standard. J Chromatogr A 961, 107–112. DOI: 10.1016/S0021-9673(02)00363-1

Chetiyanukornkul, T., Toriba, A., Kameda, T., Tang, N., Hayakawa, K., 2006. Simultaneous determination of urinary hydroxylated metabolites of naphthalene, fluorene, phenanthrene, fluoranthene and pyrene as multiple biomarkers of exposure to polycyclic aromatic hydrocarbons. Anal Bioanal Chem 386, 712–718. DOI: 10.1007/s00216-006-0628-6

Colmsjö, A., Rannug, A., Rannug, U., 1984. Some chloro derivatives of polynuclear aromatic hydrocarbons are potent mutagens in Salmonella typhimurium. Mutat Res 135, 21–29. DOI: 10.1016/0165-1218(84)90144-7

Covaci, A., Gerecke, A.C., Law, R.J., Voorspoels, S., Kohler, M., Heeb, N.V., Leslie, H.,

Allchin, C.R., De Boer, J., 2006. Hexabromocyclododecanes (HBCDs) in the environment and humans: a review. Environ Sci Technol 40, 3679–3688. DOI: 10.1021/es0602492

Crump, D., Chiu, S., Gauthier, L.T., Hickey, N.J., Letcher, R.J., Kennedy, S.W., 2011. The effects of Dechlorane Plus on toxicity and mRNA expression in chicken embryos: a comparison of in vitro and in ovo approaches. Comp Biochem Physiol C Toxicol Pharmacol 154, 129–134. DOI: 10.1016/j.cbpc.2011.04.005

de Wit, C.A., 2002. An overview of brominated flame retardants in the environment. Chemosphere 46, 583–624. DOI: 10.1016/S0045-6535(01)00225-9

Draxler, R.R. and Rolph, G.D. HYSPLIT (HYbrid Single-Particle Lagrangian Integrated Trajectory) Model access via NOAA ARL READY Website (http://www.arl.noaa.gov/HYSPLIT.php). NOAA Air Resources Laboratory, College Park, MD.

Duarte, J. H., Di Meglio, P., Hirota, K., Ahlfors, H., Stockinger, B., 2013. Differential influences of the aryl hydrocarbon receptor on Th17 mediated responses in vitro and in vivo. PloS One 8 (11), e79819. DOI: 10.1371/journal.pone.0079819

Ema, M., Fujii, S., Hirata-Koizumi, M., Matsumoto, M., 2008. Two-generation reproductive toxicity study of the flame retardant hexabromocyclododecane in rats. Reprod Toxicol 25, 335–351. DOI: 10.1016/j.reprotox.2007.12.004

EU risk assessment report., 2008. R044_0805_env_hh_final_ECB.doc < http://echa.europa.eu/documents/10162/661bff17-dc0a-4475-9758-40bdd6198f82>, accessed 11 January 2015

Fu, J., Suuberg, E.M., 2012. Thermochemical properties and phase behavior of halogenated polycyclic aromatic hydrocarbons. Environ Toxicol Chem 31, 486–493. DOI: 10.1002/etc.1709

Fujima, S., Ohura, T., Amagai, T., 2006. Simultaneous determination of gaseous and particulate chlorinated polycyclic aromatic hydrocarbons in emissions from the scorching of polyvinylidene chloride film. Chemosphere 65, 1983–1989. DOI: 10.1016/j.chemosphere.2006.07.003

Furuuchi, M., Eryu, K., Nagura, M., Hata, M., Kato, T., Tajima, N., Sekiguchi, K., Ehara, K., Seto, T., Otani, Y., 2010. Development and performance evaluation of air sampler with inertial filter for nanoparticle sampling. Aerosol Air Qual Res 10, 185–192. DOI: 10.4209/aaqr.2009.11.0070

Gauthier, L.T., Letcher, R.J., 2009. Isomers of Dechlorane Plus flame retardant in the eggs of herring gulls (Larus argentatus) from the Laurentian Great Lakes of North America: temporal changes and spatial distribution. Chemosphere 75, 115–120.

DOI: 10.1016/j.chemosphere.2008.11.030

Glynn, A., Lignell, S., Darnerud, P.O., Aune, M., Halldin Ankarberg, E., Bergdahl, I.A., Barregard, L., Bensryd, I., 2011. Regional differences in levels of chlorinated and brominated pollutants in mother's milk from primiparous women in Sweden. Environ int 37, 71–79. DOI: 10.1016/j.envint.2010.07.003

Hayakawa, K., Takatsuki, H., Watanabe, I., Sakai, S., 2004. Polybrominated diphenyl ethers (PBDEs), polybrominated dibenzo-p-dioxins/dibenzofurans (PBDD/Fs) and monobromo-polychlorinated dibenzo-p-dioxins/dibenzofurans (MoBPXDD/Fs) in the atmosphere and bulk deposition in Kyoto, Japan. Chemosphere 57, 343–356. DOI: 10.1016/j.chemosphere.2004.06.038

Hinds, W.C., 1999. Aerosol technology. 2nd ed., Wiley-Interscience, New York. ISBN: 0-471-19410-7

Hoffmann, D., Hoffmann, I., 1997. The changing cigarette, 1950-1995. J Toxicol Environ Health 50, 307–364. DOI: 10.1080/009841097160393

Hoh, E., Zhu, L., Hites, R.A., 2006. Dechlorane plus, a chlorinated flame retardant, in the Great Lakes. Environ Sci Technol 40, 1184–1189. DOI: 10.1021/es051911h

Horii, Y., Ok, G., Ohura, T., Kannanct, K., 2008. Occurrence and profiles of chlorinated and brominated polycyclic aromatic hydrocarbons in waste incinerators. Environ Sci Technol 42, 1904–1909. DOI: 10.1021/es703001f

Hu, G., Xu, Z., Dai, J., Mai, B., Cao, H., Wang, J., Shi, Z., Xu, M., 2010. Distribution of polybrominated diphenyl ethers and decabromodiphenylethane in surface sediments from Fuhe River and Baiyangdian Lake, North China. J Environ Sci 22, 1833–1839.

Hu, J., Jin, J., Wang, Y., Ma, Z., Zheng, W., 2011. Levels of polybrominated diphenyl ethers and hexabromocyclododecane in the atmosphere and tree bark from Beijing, China. Chemosphere 84, 355–360. DOI: 10.1016/S1001-0742(09)60328-4

IARC, IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, IARC, Lyon. Available from <http://monographs.iarc.fr/ENG/Classification/ClassificationsGroupOrder.pdf>, accessed 11th September, 2013.

Ishaq, R., Naf, C., Zebuhr, Y., Broman, D., Jarnberg, U., 2003. PCBs, PCNs, PCDD/Fs, PAHs and Cl-PAHs in air and water particulate samples--patterns and variations. Chemosphere 50, 1131–1150. DOI: 10.1016/S0045-6535(02)00701

Kakimoto, K., Toriba, A., Ohno, T., Ueno, M., Kameda, T., Tang, N., Hayakawa, K., 2008. Direct measurement of the glucuronide conjugate of 1-hydroxypyrene in human urine by using liquid chromatography with tandem mass spectrometry. J

Chromatogr B 867, 259–263. DOI: 10.1016/j.jchromb.2008.04.015

Kakimoto, K., Akutsu, K., Konishi, Y., Tanaka, Y., 2008a. Evaluation of hexabromocyclododecane in fish and marine mammal oil supplements. Food Chem. 107, 1724–1727. DOI: 10.1016/j.foodchem.2007.10.018

Kakimoto, K., Akutsu, K., Konishi, Y., Tanaka, Y., 2008b. Time trend of hexabromocyclododecane in the breast milk of Japanese women. Chemosphere 71, 1110–1114. DOI: 10.1016/j.chemosphere.2007.10.035

Kakimoto, K., Akutsu, K., Konishi, Y., Toriba, A., Hayakawa, K., 2012. Hexabromocyclododecane levels in foodstuff and human breast milk in Osaka, Japan. Interdisciplinary studies on environmental chemistry 6, 253–259. ISBN: 978-4-88704-157-8

Kakimoto, K., Nagayoshi, H., Konishi, Y., Kajimura, K., Ohura, T., Hayakawa, K., Toriba, A., 2014. Atmospheric chlorinated polycyclic aromatic hydrocarbons in East Asia. Chemosphere 111, 40–46. DOI: 10.1016/j.chemosphere.2014.03.072

Kang, J.H., Kim, J.C., Jin, G.Z., Park, H., Baek, S.Y., Chang, Y.S., 2010. Detection of Dechlorane Plus in fish from urban-industrial rivers. Chemosphere 79, 850–854. DOI: 10.1016/j.chemosphere.2010.02.051

Kawanishi, M., Sakamoto, M., Ito, A., Kishi, K., Yagi, T., 2003. Construction of reporter yeasts for mouse aryl hydrocarbon receptor ligand activity. Mutat Res-Gen Tox En 540, 99–105. DOI: 10.1016/S1383-5718(03)00174-8

Kim, Y.-D., Todoroki, H., Oyama, T., Isse, T., Matsumoto, A., Yamaguchi, T., Kim, H., Uchiyama, I., Kawamoto, T., 2004. Identification of cytochrome P450 isoforms involved in 1-hydroxylation of pyrene. Environ Res 94, 262–266. DOI:10.1016/S0013-9351(03)00134-8

Kim, J., Son, M.-h., Kim, J., Suh, J., Kang, Y., Chang, Y.-S., 2014. Assessment of Dechlorane compounds in foodstuffs obtained from retail markets and estimates of dietary intake in Korean population. J Hazard Mater 275, 19–25. DOI: 10.1016/j.jhazmat.2014.04.032

Kitazawa, A., Amagai, T., Ohura, T., 2006. Temporal trends and relationships of particulate chlorinated polycyclic aromatic hydrocarbons and their parent compounds in urban air. Environ Sci Technol 40, 4592–4598. DOI: 10.1021/es0602703

Krewski, D., 2009. Evaluating the effects of ambient air pollution on life expectancy. N Engl J Med 360, 413–415. DOI: 10.1056/NEJMe0809178

Kurokawa, Y., Takahiko, M., Matayoshi, N., Satoshi, T., Kazumi, F., 1998. Distribution of polychlorinated-*p*-dioxins and dibenzofurans in various sizes of air borne particles. Chemosphere 37, 2161–2171. DOI: 10.1016/S0045-6535(98)00278-1

Lee, W.-J., Wang, Y.-F., Lin, T.-C., Chen, Y.-Y., Lin, W.-C., Ku, C.-C., Cheng, J.-T., 1995. PAH characteristics in the ambient air of traffic-source. Sci Total Environ 159, 185–200. DOI: 10.1016/0048-9697(95)04323-S

Li, Y.-C., Yu, J. Z., Ho, S. S. H., Schauer, J. J., Yuan, Z., Lau, A. K. H., Louie, P. K. K., 2013. Chemical characteristics and source apportionment of fine particulate organic carbon in Hong Kong during high particulate matter episodes in winter 2003. Atmos Res 120-21, 88–98. DOI: 10.1016/j.atmosres.2012.08.005

Liu, Y., Zhu, L., Shen, X., 2001. Polycyclic aromatic hydrocarbons (PAHs) in indoor and outdoor air of Hangzhou, China. Environ Sci Technol 35, 840–844. DOI: 10.1021/es001354t

Lodovici, M., Dolara, P., Casalini, C., Ciappellano, S., Testolin, G., 1995. Polycyclic aromatic hydrocarbon contamination in the Italian diet. Food Addit Contam 12, 703–713. DOI: 10.1080/02652039509374360

Loomis, D., Grosse, Y., Lauby-Secretan, B., El Ghissassi, F., Bouvard, V., Benbrahim-Tallaa, L., Guha, N., Baan, R., Mattock, H., Straif, K., 2013. The carcinogenicity of outdoor air pollution. Lancet Oncol 14, 1262–63. DOI: 10.1016/S1470-2045(13)70487-X

Luthe, G., Stroomberg, G.J., Ariese, F., Brinkman, U.A.T., van Straalen, N.M., 2002. Metabolism of 1-fluoropyrene and pyrene in marine flatfish and terrestrial isopods. Environ Toxicol Pharmacol 12, 221–229. DOI:10.1016/S1382-6689(02)00093-5

Möller, A., Xie, Z., Sturm, R., Ebinghaus, R., 2010. Large-scale distribution of dechlorane plus in air and seawater from the Arctic to Antarctica. Environ Sci Technol 44, 8977–8982. DOI: 10.1021/es103047n

Ma, J., Horii, Y., Cheng, J., Wang, W., Wu, Q., Ohura, T., Kannan, K., 2009. Chlorinated and parent polycyclic aromatic hydrocarbons in environmental samples from an electronic waste recycling facility and a chemical industrial complex in China. Environ Sci Technol 43 (3), 643–9. DOI: 10.1021/es802878w

Ma, J., Chen, Z., Wu, M., Feng, J., Horii, Y., Ohura, T., Kannan, K., 2013. Airborne PM2.5/PM10-Associated Chlorinated Polycyclic Aromatic Hydrocarbons and their Parent Compounds in a Suburban Area in Shanghai, China. Environ Sci Technol 47, 7615–7623. DOI: 10.1021/es400338h

Ma, W.L., Liu, L.Y., Qi, H., Sun, D.Z., Shen, J.M., Wang, D.G., Li, Y.F., 2011. Dechlorane plus in multimedia in northeastern Chinese urban region. Environ Int 37, 66–70. DOI: 10.1016/j.envint.2010.07.002

Meng, X.Z., Zeng, E.Y., Yu, L.P., Guo, Y., Mai, B.X., 2007. Assessment of human exposure to polybrominated diphenyl ethers in China via fish consumption and inhalation. Environ Sci Technol 41, 4882–4887. DOI: 10.1021/es0701560

Miller, C.A., 3rd, 1999. A human aryl hydrocarbon receptor signaling pathway constructed in yeast displays additive responses to ligand mixtures. Toxicol Appl Pharmacol, 160 (3), 297–303. DOI: 10.1006/taap.1999.8769

Miranda, C. L., Chung, W. G., Wang-Buhler, J. L., Musafia-Jeknic, T., Baird, W. M., Buhler, D. R. 2006. Comparative in vitro metabolism of benzo[a]pyrene by recombinant zebrafish CYP1A and liver microsomes from ß-naphthoflavone-treated rainbow trout. Aquat Toxicol 80 (2), 101–108. DOI: 10.1016/j.aquatox.2006.07.018

Na, S., Kim, M., Paek, O., Kim, Y., 2013. Dietary assessment of human exposure to PBDEs in South Korea. Chemosphere 90, 1736–1741. DOI: 10.1016/j.chemosphere.2012.10.021

Nagayoshi, H., Kakimoto, K., Takagi, S., Konishi, Y., Kajimura, K., Matsuda, T., 2015. Benzotriazole Ultraviolet Stabilizers Show Potent Activities as Human Aryl Hydrocarbon Receptor Ligands. Environ. Sci. Technol. 49 (1), 578–587. DOI: http://dx.doi.org/10.1021/es503926w

Nilsson, U.L., Öestman, C.E., 1993. Chlorinated polycyclic aromatic hydrocarbons: method of analysis and their occurrence in urban air. Environ Sci Technol 27, 1826–1831. DOI: 10.1021/es00046a010

Ohura, T., Amagai, T., Fusaya, M., Matsushita, H., 2004. Polycyclic Aromatic Hydrocarbons in Indoor and Outdoor Environments and Factors Affecting Their Concentrations. Environ Sci Technol 38, 77–83. DOI: 10.1021/es030512o

Ohura, T., Kitazawa, A., and Amagai, T. 2004b Seasonal variability of 1-chloropyrene on atmospheric particles and photostability in toluene. Chemosphere 57, 31–837. DOI: 10.1016/j.chemosphere.2004.08.069

Ohura, T., Kitazawa, A., Amagai, T., Makino, M., 2005. Occurrence, profiles, and photostabilities of chlorinated polycyclic aromatic hydrocarbons associated with particulates in urban air. Environ Sci Technol 39, 85–91. DOI: 10.1021/es040433s

Ohura, T., Morita, M., Makino, M., Amagai, T., Shimoi, K., 2007. Aryl hydrocarbon receptor-mediated effects of chlorinated polycyclic aromatic hydrocarbons. Chem Res Toxicol 20, 1237–1241. DOI: 10.1021/tx700148b

Ohura, T., Fujima, S., Amagai, T., Shinomiya, M., 2008. Chlorinated polycyclic aromatic hydrocarbons in the atmosphere: seasonal levels, gas-particle partitioning, and origin. Environ Sci Technol 42, 3296–3302. DOI: 10.1021/es703068n

Ohura, T., Sawada, K., Amagai, T., Shinomiya, M., 2009. Discovery of novel halogenated polycyclic aromatic hydrocarbons in urban particulate matters: occurrence, photostability, and AhR activity. Environ Sci Technol 43, 2269–2275. DOI: 10.1021/es803633d

Ohura, T., Horii, Y., Kojima, M., Kamiya, Y., 2013. Diurnal variability of chlorinated polycyclic aromatic hydrocarbons in urban air, Japan. Atmos Environ 81, 84–91. DOI: 10.1016/j.atmosenv.2013.08.044

Pakalin, S., Cole, T., Steinkellner, J., Nicolas, R., Tissier, C., Munn, S., Eisenreich, S. 2007. Document available at Review on production processes of decabromodiphenyl ether (DecaBDE) used in polymeric applications in electrical and electronic equipment, and assessment of the availability of potential alternatives to DecaBDE. ISSN 1018–5593; EUR 22693 EN. European Commission <http://publications.jrc.ec.europa.eu/repository/bitstream/111111111/5259/1/EUR%22022693.pdf#search='EUR+22693'>, accessed 11 January 2015

Pinedo, J., Ibáñez, R., Lijzen, J.P.A., Irabien, Á., 2013. Assessment of soil pollution based on total petroleum hydrocarbons and individual oil substances. J Environ Manage 130, 72–79. DOI: 10.1016/j.jenvman.2013.08.048

Persistent Organic Pollutants Review Committee Sixth meeting (POPRC6). 2010. Report of the Persistent Organic Pollutants Review Committee on the work of its sixth meeting. Available from <2014.http://www.meti.go.jp/committee/summary/0004475/pdf/127_01_02_02.pdf>, accessed 11 January 2015,

Qi, H., Liu, L., Jia, H., Li, Y.F., Ren, N.Q., You, H., Shi, X., Fan, L., Ding, Y., 2010. Dechlorane Plus in surficial water and sediment in a northeastern Chinese river. Environ Sci Technol 44, 2305–2308. DOI: 10.1021/es9027106

Qiu, X., Marvin, C.H., Hites, R.A., 2007. Dechlorane plus and other flame retardants in a sediment core from Lake Ontario. Environ Sci Technol 41, 6014–6019. DOI: 10.1021/es070810b

Ren, G., Yu, Z., Ma, S., Li, H., Peng, P., Sheng, G., Fu, J., 2009. Determination of Dechlorane Plus in serum from electronics dismantling workers in South China. Environ Sci Technol 43, 9453–9457. DOI: 10.1021/es901672m

Ren, N., Sverko, E., Li, Y.F., Zhang, Z., Harner, T., Wang, D., Wan, X., McCarry, B.E., 2008. Levels and isomer profiles of dechlorane plus in Chinese air. Environ Sci Technol 42, 6476–6480. DOI: 10.1021/es800479c

Sakiyama, T., Nakano, T., 2011. The proceedings of 20th symposium on environmental chemistry held by Japan Society for Environmental Chemistry. (in Japanese). 548–549. <http://www.j-ec.or.jp/conference/20th/20_program.pdf>, accessed 05th June, 2013.

Sakiyama, T., Nakano, T., 2012. Determination of a Highly Chlorinated Flame Retardant Dechlorane Plus in Environment Samples by High-resolution GC/MS Mode.

Bunseki Kagaku 745–754. DOI: 10.2116/bunsekikagaku.61.745

Salamova, A., Hites, R.A., 2011. Dechlorane Plus in the Atmosphere and Precipitation near the Great Lakes. Environ Sci Technol 45, 9924–9930. DOI: 10.1021/es202762n

Sankoda, K., Nomiyama, K., Yonehara, T., Kuribayashi, T., Shinohara, R., 2012. Evidence for in situ production of chlorinated polycyclic aromatic hydrocarbons on tidal flats: environmental monitoring and laboratory scale experiment. Chemosphere 88, 542–547. DOI: 10.1016/j.chemosphere.2012.03.017

Sankoda K, Kuribayashi T, Nomiyama K, Shinohara R (2013) Occurrence and Source of Chlorinated Polycyclic Aromatic Hydrocarbons (Cl-PAHs) in Tidal Flats of the Ariake Bay, Japan. Environ Sci Technol 47: 7037–7044 doi: 10.1021/es3044483

Sarrión, M. N., Santos, F. J., Moyano, E., Galceran, M. T., 2003. Solid-phase microextraction liquid chromatography/tandem mass spectrometry for the analysis of chlorophenols in environmental samples. Rapid Commun Mass Spectrom 17, 39–48. DOI : 10.1002/rcm.873

She, Y.Z., Wu, J.P., Zhang, Y., Peng, Y., Mo, L., Luo, X.J., Mai, B.X., 2013. Bioaccumulation of polybrominated diphenyl ethers and several alternative halogenated flame retardants in a small herbivorous food chain. Environ Pollut 174, 164–170. DOI: 10.1016/j.envpol.2012.11.024

Shimada, T., Hayes, C. L., Yamazaki, H., Amin, S., Hecht, S. S., Guengerich, F. P., Sutter, T. R., 1996. Activation of chemically diverse procarcinogens by human cytochrome P-450 1B1. Cancer Res 56, 2979–84.

Shimada, T., Takenaka, S., Murayama, N., Yamazaki, H., Kim, J. H., Kim, D., Yoshimoto, F. K., Guengerich, F. P., Komori, M., 2015. Oxidation of acenaphthene and acenaphthylene by human cytochrome P450 enzymes. Chem Res Toxicol 28, 268–278. DOI: 10.1021/tx500505y

Shiraishi, H., Pilkington, N.H., Otsuki, A., Fuwa, K., 1985. Occurrence of chlorinated polynuclear aromatic hydrocarbons in tap water. Environ Sci Technol 19, 585–590. DOI: 10.1021/es00137a001

Siddique, S., Xian, Q., Abdelouahab, N., Takser, L., Phillips, S.P., Feng, Y.L., Wang, B., Zhu, J., 2012. Levels of dechlorane plus and polybrominated diphenylethers in human milk in two Canadian cities. Environ Int 39, 50–55. DOI:10.1016/j.envint.2011.09.010

Sohl, C. D., Isin, E. M., Eoff, R. L., Marsch, G. A., Stec, D. F., Guengerich, F. P., 2008. Cooperativity in oxidation reactions catalyzed by cytochrome P450 1A2: highly cooperative pyrene hydroxylation and multiphasic kinetics of ligand binding. J Biol Chem 283, 7293–308. DOI: 10.1074/jbc.M709783200

Strandberg, B., Dodder, N.G., Basu, I., Hites, R.A., 2001. Concentrations and spatial variations of polybrominated diphenyl ethers and other organohalogen compounds in Great Lakes air. Environ Sci Technol 35, 1078–1083. DOI: 10.1021/es001819f

Sugiyama, H., Katagiri, Y., Kaneko, M., Watanabe, T., Hirayama, T., 1999. Chlorination of pyrene in soil components with sodium chloride under xenon irradiation. Chemosphere 38, 1937–1945. DOI: 10.1016/S0045-6535(98)00407-X

Sverko, E., Tomy, G.T., Marvin, C.H., Zaruk, D., Reiner, E., Helm, P.A., Hill, B., McCarry, B.E., 2008. Dechlorane plus levels in sediment of the lower Great Lakes. Environ Sci Technol 42, 361–366. DOI: 10.1021/es0710104

Takasuga, T., Umetsu, N., Makino, T., Tsubota, K., Sajwan, K., Kumar, K., 2007. Role of Temperature and Hydrochloric Acid on the Formation of Chlorinated Hydrocarbons and Polycyclic Aromatic Hydrocarbons During Combustion of Paraffin Powder, Polymers, and Newspaper. Arch Environ Con Tox 53, 8–21. DOI: 10.1007/s00244-006-0185-1

Tang, N., Hattori, T., Taga, R., Igarashi, K., Yang, X., Tamura, K., Kakimoto, H., Mishukov, V.F., Toriba, A., Kizu, R., Hayakawa, K., 2005. Polycyclic aromatic hydrocarbons and nitropolycyclic aromatic hydrocarbons in urban air particulates and their relationship to emission sources in the Pan–Japan Sea countries. Atmos Environ 39, 5817–5826. DOI: 10.1016/j.atmosenv.2005.06.018

Tang, Y.M., Wo, Y.Y., Stewart, J., Hawkins, A.L., Griffin, C.A., Sutter, T.R., Greenlee, W.F., 1996. Isolation and characterization of the human cytochrome P450 CYP1B1 gene. J. Biol. Chem. 271, 28324–28330. DOI:10.1074/jbc.271.45.28324

Tomy, G.T., Budakowski, W., Halldorson, T., Whittle, D.M., Keir, M.J., Marvin, C., MacInnis, G., Alaee, M., 2004. Biomagnification of alpha- and gamma-hexabromocyclododecane isomers in a Lake Ontario food web. Environ Sci Technol 38, 2298–2303. DOI: 10.1021/es034968h

Tomy, G.T., Pleskach, K., Ismail, N., Whittle, D.M., Helm, P.A., Sverko, E., Zaruk, D., Marvin, C.H., 2007. Isomers of dechlorane plus in Lake Winnipeg and Lake Ontario food webs. Environ Sci Technol 41, 2249–2254. DOI:10.1021/es062781v

Toriba, A., Kitaoka, H., Dills, R. L., Mizukami, S., Tanabe, K., Takeuchi, N., Ueno, M., Kameda, T., Tang, N., Hayakawa, K., Simpson, C. D., 2007. Identification and quantification of 1-nitropyrene metabolites in human urine as a proposed biomarker for exposure to diesel exhaust. Chem Res Toxicol 20 (7), 999–1007. DOI: 10.1021/tx700015q

Tornkvist, A., Glynn, A., Aune, M., Darnerud, P.O., Ankarberg, E.H., 2011. PCDD/F, PCB, PBDE, HBCD and chlorinated pesticides in a Swedish market basket from

2005--levels and dietary intake estimations. Chemosphere 83, 193–199. DOI: 10.1016/j.chemosphere.2010.12.042

Tue, N.M., Takahashi, S., Suzuki, G., Isobe, T., Viet, P.H., Kobara, Y., Seike, N., Zhang, G., Sudaryanto, A., Tanabe, S., 2013. Contamination of indoor dust and air by polychlorinated biphenyls and brominated flame retardants and relevance of non-dietary exposure in Vietnamese informal e-waste recycling sites. Environ Int 51, 160–167. DOI: 10.1016/j.envint.2012.11.006

UK Food Standards Agency, 2006. Brominated chemicals: UK dietary intakes. Available from < http://www.food.gov.uk/science/surveillance/fsisbranch2006/fsis1006>, accessed 05th June, 2013.

US EPA, 2004. High Production Volume (HPV) Challenge Program. Available from <http://www.epa.gov/chemrtk/pubs/summaries/dechlorp/c15635tp.pdf>, accessed 05th June, 2013.

US EPA, 2009, High Production Volume (HPV) Challenge Program. <http://www.epa.gov/chemrtk/pubs/summaries/dechlorp/c15635rr3.pdf>, accessed 15th Jan 2012.

US EPA, 2011. Exposure Factors Handbook. Available from <http://www.epa.gov/ncea/efh/report.html>, accessed 05th June, 2013.

van Leeuwen, S.P., de Boer, J., 2008. Brominated flame retardants in fish and shellfish - levels and contribution of fish consumption to dietary exposure of Dutch citizens to HBCD. Mol Nutr Food Res 52, 194–203. DOI: 10.1002/mnfr.200700207

Walsh, A. A., Szklarz, G. D., Scott, E. E., 2013. Human cytochrome P450 1A1 structure and utility in understanding drug and xenobiotic metabolism. J Biol Chem 288, 12932–12943. DOI: 10.1074/jbc.M113.452953

Wang, D., Xu, X., Chu, S., Zhang, D., 2003. Analysis and structure prediction of chlorinated polycyclic aromatic hydrocarbons released from combustion of polyvinylchloride. Chemosphere 53, 495–503. DOI: 10.1016/S0045-6535(03)00507-1

Wang, D.G., Yang, M., Qi, H., Sverko, E., Ma, W.L., Li, Y.F., Alaee, M., Reiner, E.J., Shen, L., 2010. An Asia-specific source of dechlorane plus: concentration, isomer profiles, and other related compounds. Environ Sci Technol 44, 6608–6613. DOI: 10.1021/es101224y

Wang, D.G., Alaee, M., Byer, J.D., Brimble, S., Pacepavicius, G., 2013. Human health risk assessment of occupational and residential exposures to dechlorane plus in the manufacturing facility area in China and comparison with e-waste recycling site. Sci Total Environ 445–446, 329–336. DOI: 10.1016/j.scitotenv.2012.12.059

Wang, D.G., Guo, M.X., Pei, W., Byer, J. D., Wang, Z., 2015. Trophic magnification of

chlorinated flame retardants and their dechlorinated analogs in a fresh water food web. Chemosphere 118, 293–300. DOI: 10.1016/j.chemosphere.2014.09.057

Wang, J., Tian, M., Chen, S.J., Zheng, J., Luo, X.J., An, T.C., Mai, B.X., 2011. Dechlorane Plus in house dust from E-waste recycling and urban areas in South China: sources, degradation, and human exposure. Environ Toxicol Chem 30, 1965–1972. DOI: 10.1002/etc.587

Weems, J. M., and Yost, G. S. 2010. 3-Methylindole Metabolites Induce Lung CYP1A1 and CYP2F1 Enzymes by AhR and Non-AhR Mechanisms, Respectively. Chem Res Toxicol 23, 696–704. DOI: 10.1021/tx9004506

Wu, B., Liu, S., Guo, X., Zhang, Y., Zhang, X., Li, M., Cheng, S., 2012. Responses of Mouse Liver to Dechlorane Plus Exposure by Integrative Transcriptomic and Metabonomic Studies. Environ Sci Technol 46, 10758–10764. DOI: 10.1021/es301804t

Wu, J.P., Zhang, Y., Luo, X.J., Wang, J., Chen, S.J., Guan, Y.T., Mai, B.X., 2010. Isomer-specific bioaccumulation and trophic transfer of Dechlorane Plus in the freshwater food web from a highly contaminated site, South China. Environ Sci Technol 44, 606–611. DOI: 10.1021/es902744b

Xian, Q., Siddique, S., Li, T., Feng, Y.L., Takser, L., Zhu, J., 2011. Sources and environmental behavior of dechlorane plus--a review. Environ Int 37, 1273–1284. DOI: 10.1016/j.envint.2011.04.016

Yang, M., Jia, H.-L., Ma, W.-L., Qi, H., Cui, S., Li, Y.-F., 2012. Levels, compositions, and gas-particle partitioning of polybrominated diphenyl ethers and dechlorane plus in air in a Chinese northeastern city. Atmos Environ 55, 73–79. DOI: 10.1016/j.atmosenv.2012.03.040

Yang, M., Qi, H., Jia, H-L., Ren, N-Q., Ding, Y-S., Ma, W-L., Liu, L-Y., Hung, H., Sverko, E., Li, Y-F., 2013. Polybrominated Diphenyl Ethers in Air across China: Levels, Compositions, and Gas-Particle Partitioning. Environ Sci Technol 47, 8978–8984. DOI: 10.1021/es4022409

Yu, Z., Liao, R., Li, H., Mo, L., Zeng, X., Sheng, G., Fu, J., 2011. Particle-bound Dechlorane Plus and polybrominated diphenyl ethers in ambient air around Shanghai, China. Environ Pollut 159, 2982–2988. DOI: 10.1016/j.envpol.2011.04.024

Zanger, U.M., Schwab, M., 2013. Cytochrome P450 enzymes in drug metabolism: regulation of gene expression, enzyme activities, and impact of genetic variation. Pharmacol. Ther. 138, 103–141. DOI: 10.1016/j.pharmthera.2012.12.007

Zhang, H., Wang, P., Li, Y., Shang, H., Wang, Y., Wang, T., Zhang, Q., Jiang, G., 2013. Assessment on the Occupational Exposure of Manufacturing Workers to

Dechlorane Plus through Blood and Hair Analysis. Environ Sci Technol 47, 10567−10573. DOI:10.1021/es401144c

Zhang, X.L., Luo, X.J., Liu, H.Y., Yu, L.H., Chen, S.J., Mai, B.X., 2011a. Bioaccumulation of several brominated flame retardants and dechlorane plus in waterbirds from an e-waste recycling region in South China: associated with trophic level and diet sources. Environ Sci Technol 45, 400−405. DOI: 10.1021/es102251s

Zhang, Y., Wu, J.P., Luo, X.J., Sun, Y.X., Mo, L., Chen, S.J., Mai, B.X., 2011b. Biota-sediment accumulation factors for Dechlorane Plus in bottom fish from an electronic waste recycling site, South China. Environ Int 37, 1357−1361. DOI: 10.1016/j.envint.2011.06.005

Zhang, Y., Wu, J.P., Luo, X.J., Wang, J., Chen, S.J., Mai, B.X., 2011c. Tissue distribution of Dechlorane Plus and its dechlorinated analogs in contaminated fish: high affinity to the brain for anti-DP. Environ Pollut 159, 3647−3652. DOI: 10.1016/j.envpol.2011.07.026

Zheng, J., Wang, J., Luo, X.J., Tian, M., He, L.Y., Yuan, J.G., Mai, B.X., Yang, Z.Y., 2010. Dechlorane Plus in human hair from an e-waste recycling area in South China: comparison with dust. Environ Sci Technol 44, 9298−9303. DOI: 10.1021/es103105x

Zhu, J., Feng, Y.L., Shoeib, M., 2007. Detection of dechlorane plus in residential indoor dust in the city of Ottawa, Canada. Environ Sci Technol 41, 7694−7698. DOI: 10.1021/es071716y

Ziagova, M., Liakopoulou-Kyriakides, M., 2007. Comparison of cometabolic degradation of 1,2-dichlorobenzene by *Pseudomonas* sp. and *Staphylococcux xylosus*. Enzyme Microb. Technol. 40, 1244−1250. DOI: 10.1016/j.enzmictec.2006.09.004

5. List of Publication and Conference

Publications

Kakimoto K., Nagayoshi H., Inazumi N., Tani A., Konishi Y., Kajimura K., Ohura T., Nakano T., Tang N., Hayakawa K., Toriba A., “Identification and Characterization of Oxidative Metabolites of 1-Chloropyrene” Chemical research in toxicology, 28, 1728−1736 (2015)

The final publication is available at ACS Publications website

http://dx.doi.org/10.1021/acs.chemrestox.5b00173

Kakimoto, K., Nagayoshi, H., Akutsu, K., Konishi, Y., Kajimura, K., Hayakawa, K., Toriba, A., “Dechlorane Plus and decabromodiphenyl ether in atmospheric particles of northeast Asian cities” Environmental Science and Pollution Research, 122, 14600−14605 (2015)

The final publication is available at link.springer.com

http://dx.doi.org/10.1007/s11356-014-2861-4

Kakimoto, K., Nagayoshi, H., Konishi, Y., Kajimura, K., Ohura, T., Hayakawa, K., Toriba, A., “Atmospheric chlorinated polycyclic aromatic hydrocarbons in East Asia” Chemosphere, 111, 40−46 (2014)

The final publication is available at www.sciencedirect.com

http://dx.doi.org/10.1016/j.chemosphere.2014.03.072

Kakimoto, K., Nagayoshi, H., Takagi, S., Akutsu, K., Konishi, Y., Kajimura, K., Hayakawa, K., Toriba, A., “Inhalation and dietary exposure to Dechlorane Plus and polybrominated diphenyl ethers in Osaka, Japan” Ecotoxicology and Environmental Safety, 99, 69−73 (2014)

The final publication is available at www.sciencedirect.com

http://dx.doi.org/10.1016/j.ecoenv.2013.10.023

Kakimoto, K., Nagayoshi, H., Yoshida, J., Akutsu, K.,Konishi, Y., Toriba, A., Hayakawa, K., “Detection of Dechlorane Plus and brominated flame retardants in marketed fish in Japan” Chemosphere, 89, 416−419 (2012)

The final publication is available at www.sciencedirect.com

http://dx.doi.org/10.1016/j.chemosphere.2012.05.072

Kakimoto, K., Toriba, A., Ohno, T., Ueno, M., Kameda, T., Tang, N., Hayakawa, K., “Direct measurement of the glucuronide conjugate of 1-hydroxypyrene in human urine by using liquid chromatography with tandem mass spectrometry” Journal of Chromatography B, 867, 259-263 (2008)

Kakimoto, K., Akutsu, K., Konishi, Y., Tanaka, Y.. “Evaluation of hexabromocyclododecane in fish and marine mammal oil supplements” Food Chemistry, 107, 1724-1727 (2008)

The final publication is available at www.sciencedirect.com

http://dx.doi.org/10.1016/j.foodchem.2007.10.018

Kakimoto, K., Akutsu, K., Konishi, Y., Tanaka, Y., “Time trend of hexabromocyclododecane in the breast milk of Japanese women” Chemosphere, 71, 1110-1114 (2008)

The final publication is available at www.sciencedirect.com

http://dx.doi.org/10.1016/j.chemosphere.2007.10.035

6. Acknowledgements

This thesis has been produced under the direction of associate Professor Akira Toriba and Professor Kazuichi Hayakawa, Professor Akira Odani, associate Professor Yohei Sasaki at the Graduate School of Medical, Pharmaceutical and Health Sciences, Kanazawa University, and associate Professor Atsushi Matsuki at the Institute of Nature and Environmental Technology, Kanazawa University. I would like to sincerely thank them for their helpful comments and effective suggestions.

I express my sincere gratitude to associate Professor Takeshi Ohura at the Department of Environmental Bioscience, Faculty of Agriculture, Meijo University for his helpful advice and for providing me with the ClPAHs standards mixture.

I appreciate very much the support I have received from and associate Professor Ning Tang at the Institute of Nature and Environmental Technology, Kanazawa University, Office Administrator Yuka Kounishi, Chie Aoki, and all the members of the Laboratory of Hygienic Chemistry, Institute of Medical, Pharmaceutical and Health Sciences, Faculty of Pharmacy. I could not have carried out my research and prepared this thesis without their help.

I especially thank Senior Researcher Dr. Yoshimasa Konishi of the Osaka Prefectural Institute of Public Health (OPIPH) for his helpful advice and instruction, and Senior

Researcher Dr. Haruna Nagayoshi at OPIPH for her helpful advice and for AhR ligand assay experiment. I also thank Professor Takeshi Nakano at Osaka University for his dynamic and unique direction, , and Dr. Takanori Sakiyama, Osaka City Institute of Public Health and Environmental Science for his helpful comments on Dechlorane Plus, Senior Researcher Dr. Kazuhiko Akutsu at OPIPH for his helpful comments, Dr. Tahahiro Doi and Akiko Asada at OPIPH for providing me with advice about Cl-Pyr synthesis, and Dr. Keiji Kajimura, Head of the Division of Food Chemistry, OPIPH for encouraging and supporting my study on the doctoral course. I offer special thanks to Dr. Toshiki Tojo at Osaka City Institute of Public Health and Environmental Sciences for providing the filter samples of the size classified particle matter and Masami Furuuchi and Mitsuhiko Hata at Kanazawa University for helping me to use $PM_{0.1}$ air sampler, Dr. Atsushi Yamamoto at Osaka City Institute of Public Health and Environmental Sciences for providing me with advice about mass fragmentation pathway of metabolites of ClPyr, and to Assistant Professor Atsushi Tani at Osaka University for helping me on the structure determination of ClPyr metabolites.

I wish to thank all the members of the Division of Food Chemistry at OPIPH, and Dr. Sokichi Takagi, Dr. Jin Yoshida, the members of OPIPHs.

Finally, I thank my family for their constant support and encouragement.