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Assessment of human exposure to PCDDs, PCDFs and Co-PCBs using hair as a human pollution indicator sample I: development of analytical method for human hair and evaluation for exposure assessment

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Abstract

Dioxins including polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and coplanar polychlorinated biphenyls (Co-PCBs) are highly toxic. Even at environmental pollution levels, they cause hormonal damage in women, and they have been shown to induce immunosuppression and genital function damage in humans. In this study, a new method using isotope dilution was established to detect PCDDs, PCDFs and Co-PCBs in human hair. This method, comprised of washing and cutting of hair, alkaline decomposition, hexane extraction, multilayer silica gel column chromatography, high performance liquid chromatography with a porous graphite carbon column and analysis by high resolution gas chromatography/high resolution mass spectrometry, enabled us to analyze PCDDs, PCDFs and Co-PCBs at trace levels of less than pg/g with good reproducibility. In addition, there was a correlation between some isomers in human hair and blood collected from identical donors. Human hair analysis is useful to evaluate human risk assessment including that due to environmental pollution.

Keywords: Human hair; Human blood; PCDDs; PCDFs; Co-PCBs; HPLC; GC–MS

1. Introduction

Polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and coplanar polychlorinated biphenyls (Co-PCBs) are by-products of industrial and combustion processes or industrial compounds. These molecules are highly toxic and show high levels of bioaccumulation (Safe, 1987; Brunstrom, 1989; Kutz et al., 1990). These compounds have been identified as residues in various samples such as environmental materials, wildlife, human serum, adipose tissue and human milk. Moreover, preparations of human blood, human adipose tissue, human milk etc. are used to monitor human exposure. In Japan, it is possible to acquire for research purposes internal organs such as human adipose tissue and human liver after accidental death, for instance in a traffic accident. However, it is difficult to collect these organs in amounts sufficient to allow evaluation of the chemical burden by such compounds in the human body. Large blood samples of the order of 100 ml or more are required. Also, human milk can only be collected from women in a limited age range.

On the other hand, human hair can be easily collected from people over wide ranges of age, sex, residential area, eating habits and working environments. Moreover, there is no need for special apparatus for sampling of human hair. In 1991, Ohgami et al. (1991) reported

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that human hair was useful as an indicator of human exposure to pollution by PCBs and polychlorinated quaterphenyls (PCQs); that is, patients with Yusho disease caused by ingestion of rice bran oil contaminated with a PCB product (Kanechlor) in western Japan showed markedly higher levels of PCBs (28.92 ng/g) and PCQs (0.53 ng/g) in their hair than unexposed normal subjects (2.43 ng/g and <0.1 ng/g, respectively). Neuber et al. (1999) reported that hair analysis is a suitable method for detecting and quantifying indoor air pollution by POPs such as lindane and DDT. In the present study, human hair was used to evaluate arsenic and trace element pollution (Evans and Jervis, 1987; Hashimi et al., 1992; Peach and Lane, 1998). Human hair was also found to be a good indicator of the atmospheric burden of PCDDs and PCDFs (Schramm et al., 1992). In addition, human hair was reported to be an excellent material for detection of human exposure to PCDDs and PCDFs due to cigarette smoking (Schramm et al., 1993).

Therefore, hair analysis is a useful means of monitoring human exposure to PCDDs, PCDFs and non-ortho coplanar PCBs (nCo-PCBs). The aim of this study was to determine human exposure to PCDDs, PCDFs and nCo-PCBs using hair analysis. First, we established an analytical method for PCDDs, PCDFs and nCo-PCBs in human hair. Second, we analyzed hair and blood collected from six identical donors for exposure assessment.

2. Materials

Samples of about 1 kg of human hair were collected from 100 normal men at barber shops in Daitou and Hirakata cities, Osaka prefecture, Japan, in April, 1994. The hair was washed and cut in the normal manner. The hair that fell on a clean sheet around the subject's shoulders was collected and stored in a sealable polyethylene bag. The hair samples were stored at 4 °C until analysis. The samples were used for investigation of an analytical method for detection of chlorinated environmental pollutants.

For evaluation as a method for exposure assessment, individual hair and blood samples (about 100 ml) were taken from healthy six donors. Donor ages ranged from 23 to 40 years old. Blood samples were stored at 4 °C for one day. Then, the samples were centrifuged and serum was separated. Serum samples (about 35–45 ml) were stored at −20 °C until analysis.

3. Methods

3.1. Examination of hair washing frequency with a commercial shampoo

After spiking with internal standards (five $^{13}C_{12}$-PCDDs and five $^{13}C_{12}$-PCDFs, each 500 pg; three $^{13}C_{12}$-nCo-PCBs, each 1000 pg), hair samples (15 g) that were unwashed, or washed once or twice with 1% commercial shampoo were extracted with 200 ml of toluene for 4 h under reflux. After addition of keeper solvent (*n*-decane, 0.3 ml), each extract was concentrated to a volume of less than 0.3 ml and adjusted to a volume of 20 ml with *n*-hexane. The *n*-hexane solution was purified on a multilayer column containing Na_2SO_4 anhydride (4 g), 10% (w/w) $AgNO_3$–silica (4 g), silica (0.6 g), 22% (w/w) H_2SO_4–silica (3 g), 44% (w/w) H_2SO_4–silica (4 g), silica (0.6 g) and 2% (w/w) KOH–silica (2 g) with *n*-hexane as the eluent (170 ml). The eluate was concentrated to 5 ml and separated by chromatography into three fractions by successive elution with 70 ml of 2% methylene chloride in *n*-hexane and 160 ml of 50% methylene chloride in *n*-hexane on an alumina column (10 g, Merck, neutral, activate I).

After addition of keeper solvent (*n*-decane, 10 μl), the third eluate containing nCo-PCBs and PCDDs/PCDFs was concentrated and then adjusted to a volume of 10 μl with *n*-decane.

The purified extract was analyzed on a Supelco SP-2331 capillary column (60 m × 0.32 mm, 0.20 μm) (held for 1 min at 140 °C, programmed for 140–200 °C at 10 °C/min and to 255 °C at 3.5 °C/min, and held for 13 min) for tetra- to hexachlorinated PCDDs and PCDFs, on a J&W DB-5 capillary column (30 m × 0.32 mm, 0.25 μm) (held for 1 min at 140 °C, programmed for 140–220 °C at 20 °C/min and to 310 °C at 8 °C/min, and held for 2 min) for hepta- and octachlorinated PCDDs and PCDFs, and on a J&W DB-5 capillary column (30 m × 0.32 mm, 0.25 μm) (held for 1 min at 120 °C, programmed for 120–180 °C at 20 °C/min and to 250 °C at 4° C/min, and to 310 °C at 20 °C/min) for nCo-PCBs, in electron impact-selected ion monitoring mode at a resolution of 7000–10 000 using a Hewlett Packard 5890J gas chromatograph (HRGC)-JEOL SX-102 mass spectrometer (HRMS).

3.2. Examination of extraction time

After spiking with internal standards, samples ≈5 mm in length (90 g) were extracted with 500 ml of toluene for 1 h. After completely removing the extract, the same amounts of the internal standards and toluene were added to the original hair sample and extracted for 2 h. The same trial was repeated four more times. Each extract was analyzed according to the above method.

3.3. Examination of reproducibility of analytical data obtained by extraction with toluene under reflux

After spiking with internal standards, five hair samples of 15 g each were extracted with 200 ml of toluene for 3 h under reflux. Each extract was analyzed ac-

cording to the method described above. The coefficient of variation (CV) for each dioxin was calculated from the analytical data.

3.4. Examination of extraction efficiency

Hair samples (15 g), which were cut into lengths of ≈5 and 1 mm, were prepared for comparison of extraction efficiency. Hair samples (15 g) ≈1 mm in length were powdered using sea sand (45 g). Each sample was analyzed according to the method described above.

3.5. Examination of hair decomposition under various alkaline conditions

Hair samples (1 g) and alkaline solution (8 ml) were added to test tubes (10 ml), which were then mechanically shaken at room temperature. The conditions of alkaline solution and shaking time are given in Table 3. After shaking, the solubility of the hair was checked.

3.6. Examination of stability of OCDD in various alkaline solutions

Aliquots of 10 μl of 1 mg/l OCDD solution were poured into test tubes containing 4 ml of each alkaline solution described in Fig. 3. The test tubes were mechanically shaken for a period of 0.5–4 h at room temperature. After shaking, the solution was extracted twice with 4 ml of *n*-hexane. The extract was washed with water, dried on anhydrous sodium sulfate, concentrated, dissolved with 100 μl of *n*-decane and analyzed for OCDD using a gas chromatograph with ^{63}Ni-ECD.

3.7. Search for impurities in dioxin analysis

As impurities were detected on dioxin analysis, we searched for impurities by HRGC/low resolution mass spectrometry (LRMS).

3.8. Development of purification method

The impurities were not separated on alumina column chromatography performed according to the standard procedure. Therefore, we attempted to purify these impurities by high performance liquid chromatography (HPLC) with a porous graphitic carbon (PGC) column.

3.9. Examination of reproducibility of analytical data with alkaline extraction and comparison of reflux extraction with alkaline extraction

After spiking with internal standards, three hair samples of 15 g were decomposed in 100 ml of 2 N KOH/H_2O solution for 4 h at room temperature. The alkaline solution was extracted twice with *n*-hexane. The extract was washed, dried over anhydrous Na_2SO_4, concentrated and cleaned up on a multi-layer column, followed by HPLC with a PGC column. The purified extracts were analyzed for nCo-PCBs, PCDDs and PCDFs using HRGC-HRMS as described above.

3.10. Examination of correlation between levels in hair and blood to estimate body burdens or special exposure

3.10.1. Lipid content

After addition of potassium oxalate (0.25 g), 10 g of serum was shaken with 25 ml of ethanol, 50 ml of diethyl ether and 50 ml of petroleum ether.

After separation of the organic phase, the aqueous phase was extracted with 50 ml of diethyl ether/petroleum ether (1:1). The combination of the first and second extracts was washed twice with 250 ml of 2% saline solution, followed by washing twice with 100 ml of water. After evaporating to dryness over anhydrous sodium sulfate, the washed extract was concentrated and the remaining solvent was completely evaporated. The lipid content of serum was calculated on the basis of the gained lipid weight.

3.10.2. Analysis of blood sample

After addition of $^{13}C_{12}$-labeled internal standards of PCDDs, PCDFs and nCo-PCBs, 30–35 g of serum was prepared by mechanical shaking at room temperature for 2 h with 2 N KOH/ethanol 50 ml. After addition of 50 ml of 10% saline solution, the treated solution was extracted twice with 30 ml of *n*-hexane, followed by washing twice with 30 ml of water. The extract was concentrated and cleaned up on a 10% (w/w) $AgNO_3$–silica (1 g) column with *n*-hexane as the eluent (10 ml). The eluate solution was loaded on to an active carbon silica gel column (0.1 g), and separated into two fractions. The first fraction containing PCBs (except for nCo-PCBs) was eluted with 5 ml of *n*-hexane and 25% methylene chloride in *n*-hexane. PCDDs, PCDFs and nCo-PCBs were eluted with 30 ml of toluene as the second fraction. The purified extract was analyzed on a J&W DB-17 capillary column (30 m × 0.32 mm, 0.25 μm) (held for 1.5 min at 140 °C, programmed for 140–260 °C at 20 °C/min and to 280 °C at 3 °C/min, and held for 8 min) for tetra- to octachlorinated PCDDs and PCDFs, on a SGE HT-8 capillary column (50 m × 0.22 mm, 0.25 μm) (held for 1 min at 130 °C, programmed for 130–220 °C at 20 °C/min and to 320 °C at 5 °C/min, and held for 5 min) for tetra- and hexachlorinated nCo-PCBs, using HRGC-HRMS as described above.

4. Results and discussion

In general, environmental chlorinated pollutants in human hair are considered to be deposited via two routes, ingestion and atmospheric deposition (Schramm et al., 1992, 1993). This is very important for assessment of human exposure to such pollutants using hair samples. Therefore, we developed an analytical procedure for PCDDs, PCDFs and nCo-PCBs in human hair.

Although, extraction of hair samples has been carried out by soxhlet extraction with toluene (Schramm et al., 1992), we used reflux extraction with toluene. From our earlier studies, this under reflux extraction shows excellent extraction time and extraction efficiency.

As shown in Fig. 1, washing once with common surfactant markedly decreased the levels of PCDDs and PCDFs in hair samples by 50% and 64%, respectively. Washing once more, however, had no further effect on the elimination of either chemical. In addition, both the unwashed and washed samples contained similar compositions of PCDDs and PCDFs.

These results suggested that PCDDs and PCDFs on the hair surface are deposited mainly via atmospheric transfer, and are completely removed from the surface of the hair by the first wash. The residual amounts of these compounds were considered to be contained in the inner part of the hair.

We next determined a suitable extraction time. Table 1 shows the amounts of PCDDs, PCDFs and nCo-PCBs extracted from hair samples during each extraction time with toluene under reflux. A large proportion of the amount of all compounds extracted was derived from the first 1 h extraction. For example, the levels of 1,2,3,4,6,7,8-HpCDD, OCDD, 1,2,3,4,6,7,8-HpCDF and 3,3′,4,4′-TCB were 95%, 96%, 93% and 93%, respectively. No observable amounts of any of the compounds were

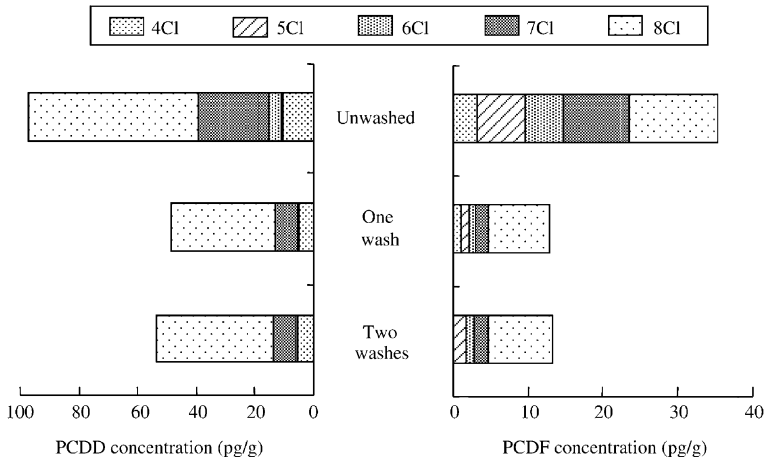

Fig. 1. Effects of contamination levels of PCDDs and PCDFs in human hair by washing with common surfactant.

Table 1
Amounts of main components extracted from a sample of human hair during a period of each extraction time with toluene under reflux

Extraction time (h)	Concentration (pg/g)				
	HpCDD	OCDD	OCDF	TCB	PeCB
0–1	18.0 (92)	290 (96)	1.00 (100)	240 (93)	25.0 (94)
1–3	1.50 (8.0)	11.0 (4.0)	<0.250	18.0 (7.0)	1.50 (6.0)
3–5	<0.120	<0.250	<0.250	<0.250	<0.300
5–7	<0.120	<0.250	<0.250	<0.250	<0.300
7–9	<0.120	<0.250	<0.250	<0.250	<0.300
9–12	<0.120	<0.250	<0.250	<0.250	<0.300
12–24	<0.120	<0.250	<0.250	<0.250	<0.300

HpCDD: 1,2,3,4,6,7,8-HpCDD, HpCDF: 1,2,3,4,6,7,8-HpCDF, OCDD: 1,2,3,4,6,7,8,9-OCDD, OCDF: 1,2,3,4,6,7,8,9-OCDF, TCB: 3,3′,4,4′-TCB, PeCB: 3,3′,4,4′,5-PeCB.

detected in the period from 3 to 5 h. Consequently, 3 h was concluded to be a suitable extraction time.

Table 2 shows reproducibility of the quantitative results of five analyses using the extraction method with toluene under reflux. The concentrations of the main components of PCDDs, PCDFs and nCo-PCBs in hair samples are shown along with their standard deviations (SD) and CV. The SD ranged between 0.333% and 3.40%, and CV ranged between 18.7% and 206%. This large CV range of extraction efficiency was estimated to be due to the hair length. Therefore, we next examined whether hair length affected extraction efficiency of PCDDs, PCDFs and nCo-PCBs.

Fig. 2 shows a comparison of extraction efficiency with regard to hair length, with values of 1 mm (powdered hair) and 5 mm. Extraction efficiencies of PCDDs, PCDFs and nCo-PCBs in powdered hair were greater than those in samples 5 mm in length. The levels of 1,2,3,4,6,7,8-HpCDD, OCDD, OCDF, 3,3′,4,4′-TCB and 3,3′,4,4′,5-PeCB, the main components in hair samples, were 15.8, 92.4, 3.14, 69.0 and 6.69 pg/g, respectively. The ratio of extraction efficiency of powdered hair versus that of 5 mm long hair samples ranged between 1.64 and 22.3. These observations clearly indicated that higher extraction efficiency was achieved with shorter hair length. The pollutants present within the hair are difficult to extract through the hard cuticle of the hair. Therefore, the development of a new analytical method was necessary, because it was difficult to make powdered hair 1 mm or less in length. Consequently, we examined the alkaline decomposition extraction method of hair samples based on the above results.

Table 3 shows the solubility of hair under various conditions, with −, +, ++, ++(+) and +++ roughly indicating solubilities of 0%, 10%, 50%, 90% and 100%, respectively. In the case of 2 N alkaline solution, the whole hair sample was dissolved in only KOH/H_2O with 4 h of mechanical shaking. However, complete

Table 2
Reproducibility of analytical data ($n = 5$) using the reflux extraction method

Compounds	Average (pg/g) ± SD	CV (%)
2,3,7,8-TCDD	4.35 ± 3.40	75.0
1,2,3,7,8-PeCDD	0.600 ± 0.782	130
1,2,3,4,7,8-HxCDD	1.03 ± 1.12	109
1,2,3,6,7,8-HxCDD	2.14 ± 2.24	104
1,2,3,7,8,9-HxCDD	0.342 ± 0.469	137
1,2,3,4,6,7,8-HpCDD	0.928 ± 0.333	35.9
OCDD	8.17 ± 1.53	18.7
2,3,7,8-TCDF	2.56 ± 3.35	131
1,2,3,7,8-PeCDF	0.394 ± 0.421	107
2,3,4,7,8-PeCDF	0.462 ± 0.702	152
1,2,3,4,7,8-HxCDF	0.242 ± 0.260	108
1,2,3,6,7,8-HxCDF	0.354 ± 0.385	109
1,2,3,7,8,9-HxCDF	0.254 ± 0.384	151
2,3,4,6,7,8-HxCDF	0.342 ± 0.253	74.0
1,2,3,4,6,7,8-HpCDF	1.82 ± 1.18	67.4
1,2,3,4,7,8,9-HpCDF	0.290 ± 0.599	206
OCDF	1.91 ± 2.22	116
3,4,4′,5-TCB	NA[a]	–
3,3′,4,4′-TCB	7.45	–
3,3′,4,4′,5-PeCB	0.300	–
3,3′,4,4′,5,5′-HxCB	ND[b]	–

Detection limit—TCDD/F–HxCDD/F: 0.088–0.10 pg/g; HpCDD/F: 0.12 pg/g; OCDD/F: 0.25 pg/g; Co-PCBs: 0.25–0.38 pg/g.

[a] NA: not analyzed.

[b] ND: not detected.

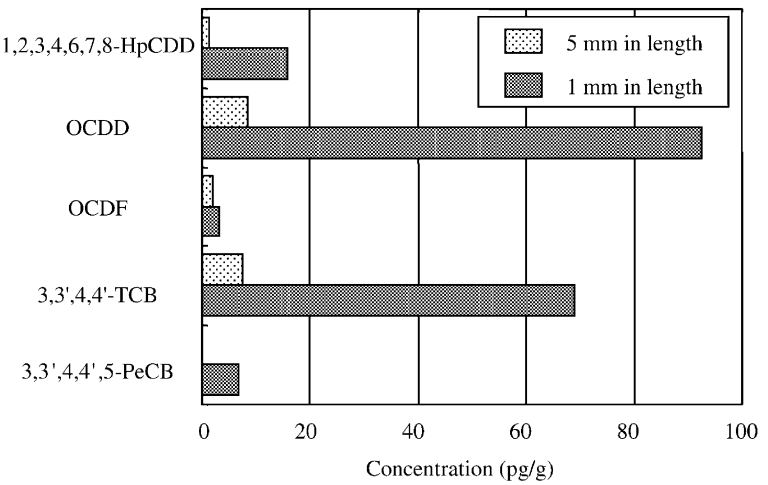

Fig. 2. Comparison of extraction efficiency.

Table 3
Solubility of human hair under various alkaline conditions

	1 h	2 h	3 h	4 h
3 N KOH/ethanol	++	+++	+++	+++
2 N KOH/ethanol	+	++	++(+)	++(+)
3 N KOH/H_2O	+	++(+)	+++	+++
2 N KOH/H_2O	+	++	++	+++
1 N KOH/H_2O	–	++	++	++
3 N NaOH/ethanol	++	++(++)	+++	+++
3 N NaOH/H_2O	+	++	+++	+++
2 N NaOH/H_2O	–	++	++(+)	++(+)

–: insoluble; +: 30% soluble; ++: 70% soluble; ++(+): 90% soluble; +++: completely soluble.

dissolution failed in 1 N KOH/H_2O. On the other hand, all 3 N alkaline solutions decomposed hair completely during a shorter treatment period of 3 h.

PCB congeners are stable in hot alkaline solution. Ryan et al. (1989) reported that highly chlorinated PCDD and PCDF congeners, however, were readily

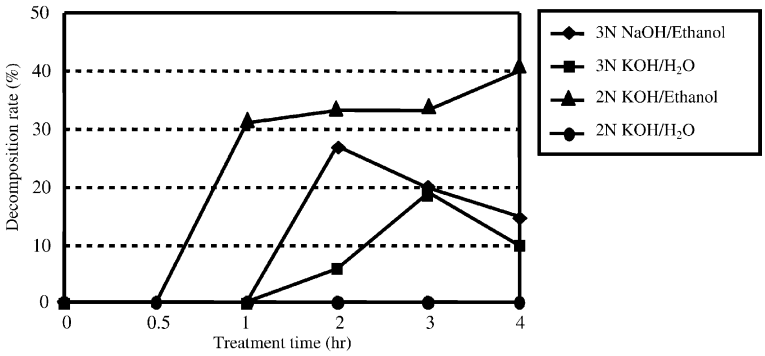

Fig. 3. Decomposition of OCDD under various alkaline conditions.

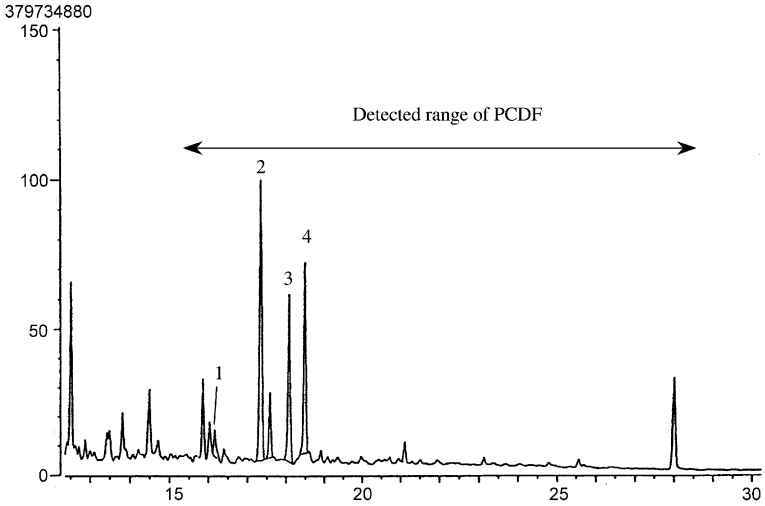

Fig. 4. HRGC/LRMS total ion chromatogram of PCDD and PCDF fractions from human hair.

decomposed by hot alkaline treatment. The degradation rate of OCDD was the highest among all congeners tested. Therefore, we examined the stability of OCDD by mechanical shaking with various alkaline solutions at room temperature. As shown in Fig. 3, we examined the stability of OCDD in all four solutions of 3 N NaOH/ethanol (EtOH), 3 N KOH/H_2O, 2 N KOH/EtOH and 2 N KOH/H_2O with shaking for 4 h. However, 2 N KOH/EtOH decomposed ≈30% of OCDD within 1 h. With treatment for more than 2 h, only 2 N KOH/H_2O showed no adverse effect on OCDD. Degradation ability was in the order 2 N KOH/EtOH > 3 N NaOH/EtOH > 3 N KOH/H_2O > 2 N KOH/H_2O. Based on the results of hair solubility and OCDD degradation, we selected 2 N KOH/H_2O as a suitable solution for analysis of PCDDs, PCDFs and nCo-PCBs in human hair.

We dissolved hair samples by the above method and extracted pollutants by liquid–liquid partitioning with *n*-hexane. The extract was purified by the standard method, i.e. multi-layer column and alumina column chromatography. Finally, the purified extract was analyzed for PCDDs, PCDFs and nCo-PCBs. However, extraction by the standard method was unable to purify some impurities in the mass range from tetra- to heptachlorinated congeners of PCDF. Therefore, we searched for these impurities by HRGC/LRMS. Fig. 4 shows the results of HRGC–LRMS total ion chromatography.

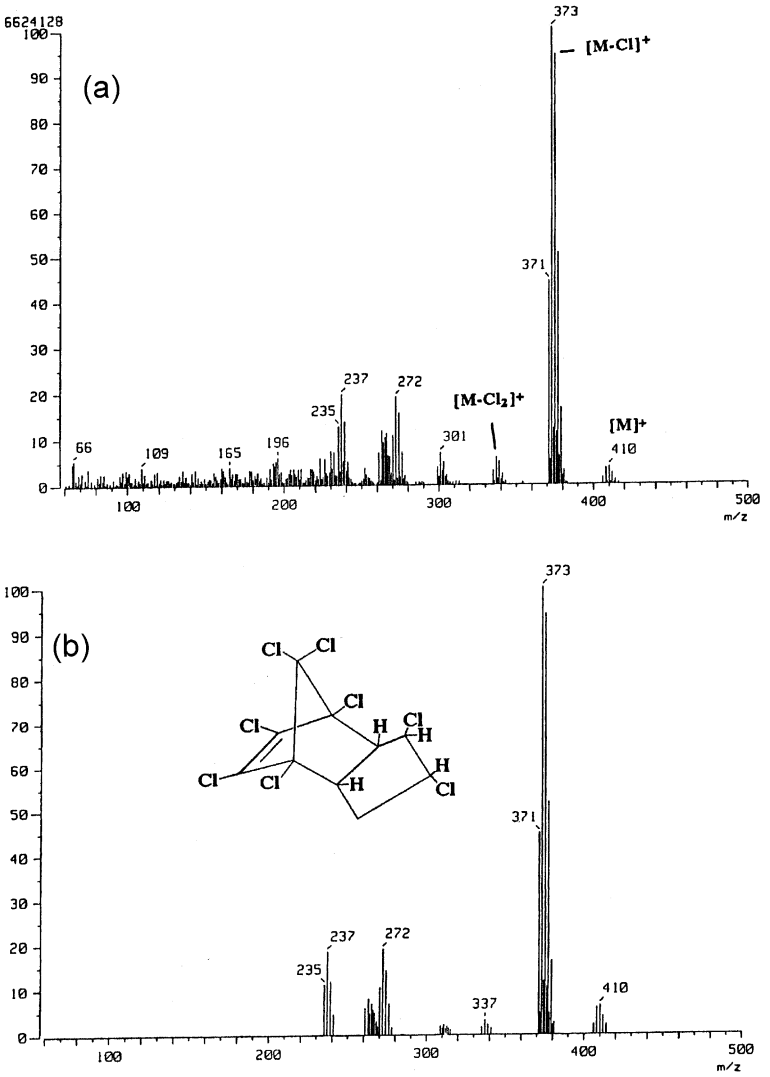

Fig. 5. (a) Mass spectrum of Peak No. 2 shown in the total ion chromatogram. (b) Standard mass spectrum of *trans*-chlordane.

The masses of these impurities (Peak No. 1–4) were similar to those of PCDFs, and it was these impurities that interfered with dioxin analysis. Fig. 5a shows the mass spectrum obtained for one of these impurity peaks (Peak No. 2) together with the standard mass spectrum of *trans*-chlordane. The peak at m/z 406–414 is a parent ion cluster, and the homologue ratio agreed with an octachlorinated compound. We also detected the characteristic fragment ion $[M–Cl]^+$ produced by desorption of a chlorine atom from the parent ion $[M]^+$. Moreover, $[M–2Cl]^+$ with desorption of a chlorine atom from $[M–Cl]^+$ was also detected. It was clear from this cleavage pattern that one of these impurities was *trans*-chlordane in agreement with the mass spectrum and retention time of standard *trans*-chlordane (Fig. 5b). Consequently, the following impurities were identified based on their mass spectra and retention times: Peak No. 1, heptachlor; Peak No. 3, *cis*-chlordane; Peak No. 4, nonachlor.

It is not possible to separate chlordanes and dioxins by multi-layer column and alumina column chromatography. Planar aromatic compounds including dioxins have been adsorbed strongly in PGC because they have a planar structure (Seriano et al., 1991; Hong et al., 1992; Takasuga et al., 1993). On the other hand, chlordanes were separated using HPLC with a PGC column under the conditions shown in Table 4 because it has a non-planar structure such as chlordanes. Therefore, this method using HPLC with a PGC column showed excellent separation of planar compounds.

Table 4
HPLC conditions for purification of impurities in human hair

Apparatus	Shimadzu LC-6A
Column	Shandon Hypercarb PCB, PGC 50 mm (length) × 4.6 mm (ID) 7 μm (particle diameter)
Column heater	60 °C
Injection volume	50 μl
Detector	UV-254 nm
Mobile phase	Fraction 1: *n*-hexane 0–3.5 ml Chlordanes and PCBs other than mCo- and nCo-PCBs Fraction 2: 1% toluene/*n*-hexane 3.5–8.5 ml mCo-PCBs Fraction 3: 50% toluene/*n*-hexane 8.5–15.5 ml nCo-PCBs Fraction 4: toluene reverse flow 15.5–23.5 ml PCDDs and PCDFs

Table 5 shows the levels of PCDDs, PCDFs and nCo-PCBs, SD and CV, and their ratio (alkaline decomposed extraction/toluene under reflux extraction). The reproducibility and extraction efficiency were very good. Therefore, for analysis of human hair the opti-

Table 5
Reproducibility of analytical data ($n = 3$) using the alkaline extraction method

Compounds	Average (pg/g) ± SD	CV (%)	Ratio[a]
2,3,7,8-TCDD	0.902 ± 0.108	12.0	0.199
1,2,3,7,8-PeCDD	0.381 ± 0.055	14.3	0.635
1,2,3,4,7,8-HxCDD	0.362 ± 0.069	19.0	0.351
1,2,3,6,7,8-HxCDD	1.05 ± 0.143	13.6	0.491
1,2,3,7,8,9-HxCDD	0.454 ± 0.125	27.5	1.33
1,2,3,4,6,7,8-HpCDD	4.08 ± 0.182	4.47	4.40
OCDD	224 ± 56.9	25.4	27.4
2,3,7,8-TCDF	1.56 ± 0.139	8.86	0.609
1,2,3,7,8-PeCDF	1.16 ± 0.196	16.9	2.94
2,3,4,7,8-PeCDF	1.33 ± 0.198	14.9	2.88
1,2,3,4,7,8-HxCDF	1.33 ± 0.093	7.00	5.50
1,2,3,6,7,8-HxCDF	1.17 ± 0.092	7.84	3.31
1,2,3,7,8,9-HxCDF	0.287 ± 0.181	62.9	1.13
2,3,4,6,7,8-HxCDF	1.83 ± 0.070	3.84	5.35
1,2,3,4,6,7,8-HpCDF	4.07 ± 0.315	7.75	2.24
1,2,3,4,7,8,9-HpCDF	0.283 ± 0.021	7.31	0.976
OCDF	3.52 ± 0.124	3.52	1.84
3,4,4′,5-TCB	16.3 ± 1.10	6.79	–
3,3′,4,4′-TCB	78.5 ± 0.960	1.22	10.5
3,3′,4,4′,5-PeCB	5.38 ± 0.348	6.46	17.9
3,3′,4,4′,5,5′-HxCB	1.34 ± 0.039	2.90	–

Detection limit—TCDD/F–HxCDD/F: 0.088–0.10 pg/g; HpCDD/F: 0.12 pg/g; OCDD/F: 0.25 pg/g; Co-PCBs: 0.25–0.38 pg/g.
[a] Ratio of levels of dioxin analogues with alkaline decomposed extraction method versus the reflux extraction method.

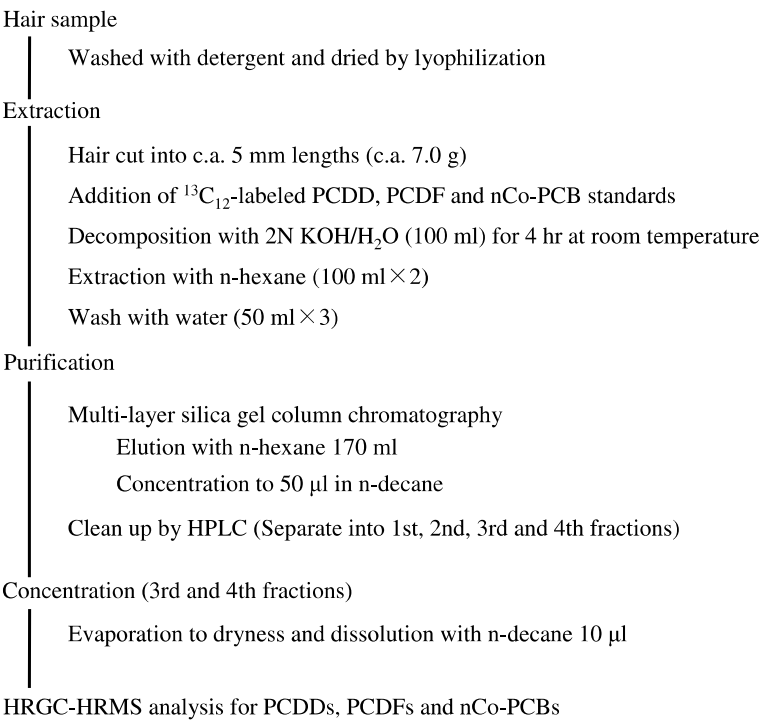

Fig. 6. Outline of analytical procedure for PCDDs, PCDFs and nCo-PCBs in human hair.

mal method was a combination of refinement by alkaline decomposition, liquid–liquid partition extraction with *n*-hexane, multi-layer silica gel column chromatography, HPLC with a PGC column and HRGC–HRMS. As shown in Fig. 6, we developed a new analytical method for PCDDs, PCDFs and nCo-PCBs in human hair.

Table 6 shows the levels of PCDDs, PCDFs and nCo-PCBs in human hair and blood collected from six identical healthy donors. As mentioned above, the main components were 1,2,3,4,6,7,8-HpCDD, OCDD, OCDF, 3,3′,4,4′-TCB, and 3,3′,4,4′,5,5′-PeCB in human hair; the levels of OCDD and 3,3′,4,4′-TCB levels were especially high. The levels ranged from 17.4 to 97.7 pg/g for OCDD, and 17.2 to 46.1 pg/g for 3,3′,4,4′-TCB. The isomers detected in blood agreed frequently with components of hair, and 1,2,3,4,6,7,8-HpCDD, OCDD, 3,3′,4,4′-TCB and 3,3′,4,4′,5,5′-PeCB were detected in common. 1,2,3,6,7,8-HxCDD and 3,3′,4,4′,5,5′-HxCB were only detected in blood samples. 2,3,7,8-Chlorine-substituted isomers were detected in both hair and blood. On the other hand, the isomers detected in human hair were not only 2,3,7,8-chlorine-substituted isomers but also other isomers (Fig. 7). Human blood was detected only 2,3,7,8-chlorine-substituted isomers. Fig. 7 shows the contributions of the main components and other components in human hair and blood collected from Donor 2. OCDD was the main component in human hair and blood, whereas the ratio (15%) of others in blood sample was small.

Van den Berg et al. (1986) reported that all 2,3,7,8-chlorine-substituted isomers were retained in the liver, following administration of fly ash extract to rats and guinea pigs. Most 2,3,7,8-chlorine-substituted PCDDs and PCDFs were detected in blood samples, in agreement with the results reported by Van den Berg et al. (1986). Interestingly, human hair showed partially similar results, but non-2,3,7,8-chlorine-substituted PCDDs and PCDFs were detected at high concentrations. We estimated that accumulation of PCDDs, PCDFs and nCo-PCBs to human hair was due not only to body burden but also special exposure such as atmospheric burden.

2,3,7,8-TCDD toxic equivalent (TEQ) levels ranged between 1.18 and 3.26 pgTEQ/g in human hair, and between 12.8 and 51.2 pgTEQ/g in blood (Table 6). Total TEQ concentrations of individual donors showed no correlation between human hair and blood. However, despite this observation, correlations between hair and blood for each isomer of PCDDs, PCDFs and nCo-PCBs were examined. The correlation factors between hair and blood ($n = 6$) of 1,2,3,4,7,8-HxCDD and 2,3,4,7,8-PeCDF were 0.626 and 0.926, respectively. The other isomers showed no correlations.

Table 6
Actual concentrations (pg/g) and total TEQ concentrations (pgTEQ/g) of PCDDs, PCDFs and nCo-PCBs in human hair and blood serum collected from six healthy donors

Compounds	Donor no. sex/age											
	Donor 1 male/23		Donor 2 male/25		Donor 3 male/38		Doner 4 male/40		Donor 5 female/23		Donor 6 female/35	
	Hair	Blood	Hair	Blood	Hair	Blood	Hair	Blood	Hair	Blood	Hair	Blood
2,3,7,8-TCDD	0.563	ND	1.05	1.43	0.494	3.41	0.164	3.24	0.342	1.93	0.307	2.22
1,2,3,7,8-PeCDD	0.540	8.36	0.519	3.10	0.363	9.85	0.123	9.55	0.320	6.38	0.878	14.1
1,2,3,4,7,8-HxCDD	1.66	3.50	1.46	2.35	0.830	4.16	ND	ND	1.47	4.01	1.96	7.69
1,2,3,6,7,8-HxCDD	0.831	29.7	0.536	15.5	0.951	54.8	0.636	33.3	1.10	36.0	0.747	58.9
1,2,3,7,8,9-HxCDD	0.376	3.21	0.293	3.37	ND	7.05	ND	6.04	0.119	7.23	ND	5.96
1,2,3,4,6,7,8-HpCDD	2.82	21.9	3.00	11.4	5.07	36.2	2.74	8.77	5.94	20.9	6.78	19.3
OCDD	24.8	71.8	17.4	115	97.7	206	33.7	150	50.8	220	34.4	440
Total PCDDs (TEQ)	1.46	13.0	1.95	6.78	1.22	20.1	0.481	16.9	1.04	13.3	1.54	23.8
2,3,7,8-TCDF	0.393	4.63	0.289	ND	0.923	ND	0.713	ND	0.364	1.56	1.03	ND
1,2,3,7,8-PeCDF	0.491	1.77	0.539	ND	0.388	ND	0.221	ND	0.534	ND	1.11	ND
2,3,4,7,8-PeCDF	0.497	ND	0.384	4.95	0.538	15.4	0.356	10.1	0.621	11.1	1.67	31.1
1,2,3,4,7,8-HxCDF	0.971	3.41	0.732	4.65	0.476	6.51	0.163	5.91	0.836	5.48	0.646	11.7
1,2,3,6,7,8-HxCDF	0.242	6.55	ND	4.93	0.166	8.48	ND	7.02	0.617	5.84	ND	20.4
1,2,3,7,8,9-HxCDF	0.774	ND	0.588	ND	0.388	5.61	0.158	ND	0.540	ND	1.06	ND
2,3,4,6,7,8-HxCDF	0.601	8.05	0.387	2.96	0.397	ND	0.843	ND	0.971	2.39	0.763	10.2
1,2,3,4,6,7,8-HpCDF	1.49	ND	1.08	6.28	1.34	14.3	0.753	2.72	24.4	4.91	3.25	13.2
1,2,3,4,7,8,9 HpCDF	0.207	ND	ND	ND	0.031	ND	0.239	ND	0.810	ND	0.560	ND
OCDF	4.93	5.71	2.55	ND	1.64	36.6	0.728	ND	65.5	ND	2.46	ND
Total PCDFs (TEQ)	0.627	6.39	0.481	4.09	0.570	11.9	0.403	6.78	1.51	7.23	1.30	20.2
3,4,4′,5-TCB (#81)	1.90	ND	2.11	ND	2.81	8.90	3.29	ND	1.22	ND	2.57	ND
3,3′,4,4′-TCB (#77)	25.7	12.0	28.3	ND	42.0	95.0	46.1	ND	17.2	ND	42.3	ND
3,3′,4,4′,5-PeCB (#126)	3.43	57.5	3.23	17.1	4.41	99.0	2.85	69.3	2.69	33.5	3.98	61.8
3,3′,4,4′,5,5′-HxCB (#169)	0.810	49.8	0.423	17.0	1.09	96.0	0.374	71.6	0.714	34.5	1.05	95.0
Total Co-PCBs (TEQ)	0.354	6.25	0.330	1.88	0.456	10.9	0.294	7.65	0.278	3.70	0.413	7.13
Total PCDDs/DFs (TEQ)	2.10	19.4	2.43	10.9	1.79	32.0	0.883	23.7	2.55	20.5	2.85	44.0
Total PCDDs/DFs/Co-PCBs (TEQ)	2.45	25.6	2.76	12.8	2.25	42.8	1.18	31.4	2.83	24.2	3.26	51.2

pg/g; pgTEQ/g—hair: on dry weight basis; blood: on lipid weight basis; ND: not detected.
Detection limit—(hair) TCDD/F—HxCDD/F: 0.088–0.10 pg/g, HpCDD/F: 0.12 pg/g, OCDD/F: 0.25 pg/g, nCo-PCBs: 0.25–0.38 pg/g; (blood)—TCDD/F–HxCDD/F: 0.53–2.2 pg/g, HpCDD/F: 2.3 pg/g, OCDD/F: 1.7 pg/g, nCo-PCB: 3.9–5.0 pg/g.

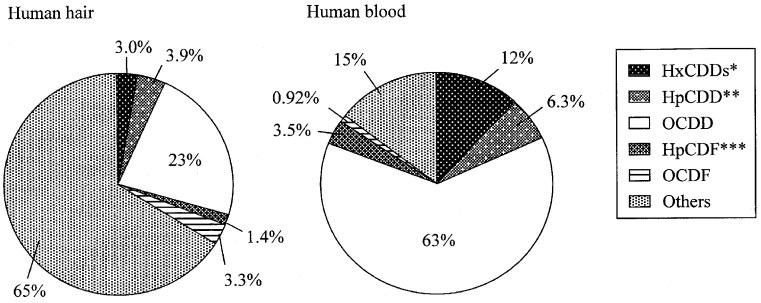

Fig. 7. Congeners contributions of main components and other congeners in human hair and blood samples collected from Donor 2. *HxCDDs: 1,2,3,4,7,8-, 1,2,3,6,7,8-HxCDD, **HpCDD: 1,2,3,4,6,7,8-HpCDD, ***HpCDF: 1,2,3,4,6,7,8-HpCDF.

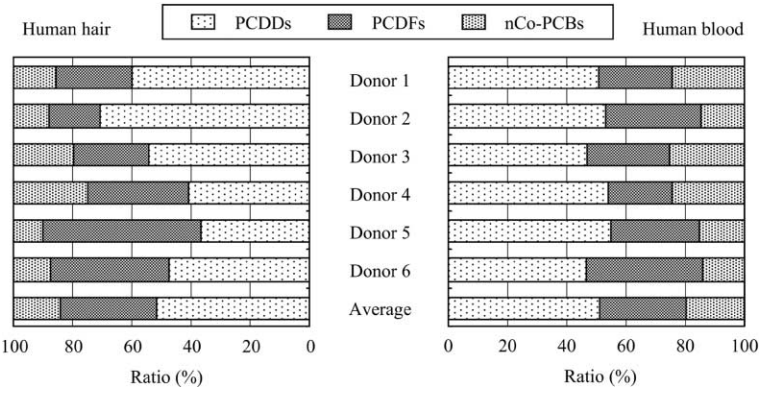

Fig. 8. Contributions of TEQ levels for PCDDs, PCDFs and nCo-PCBs in human hair and blood.

Moreover, there were no correlations for the two isomers 3,3′,4,4′-TCB and 3,4,4′,5-TCB. 3,3′,4,4′,5-PeCB and 3,3′,4,4′,5,5′-HxCB isomers were correlated between hair and blood, 0.658 and 0.667, respectively.

Fig. 8 shows comparison of composition ratios of TEQ levels for PCDDs, PCDFs and nCo-PCBs in human hair and blood. With the exception of Donor 5, the contributions were similar for all donors, with ratios of PCDDs, PCDFs and nCo-PCBs of 50%, 30% and 20%, respectively. The contribution in human hair often agreed with that in the blood. The PCDF contribution of Donor 5 was also high, and was considered to have been influenced by the outside environment.

In conclusion, our results indicated that the hair analysis was very useful for human risk assessment, because some isomers reflected human body burden, such as the blood level. Also, we concluded that PCDD, PCDF and nCo-PCB levels in hair reflect those in the environment.

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