ANALYTICAL METHOD FOR POLYCHLORINATED DIBENZO-P-DIOXINS, POLYCHLORINATED DIBENZOFURANS AND NON-ORTHO CHLORINE SUBSTITUTED COPLANAR PCBS IN HUMAN HAIR

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1. INTRODUCTION

It was revealed that the hair levels of PCBs and polychlorinated quaterphenyls (PCQs) reflected their body burden of Yusho patients[1]. Regarding toxic polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs), the human hair was also found to be a good indicator sample of their atmospheric burden to human being[2]. In addition, the hair sample was reported an excellent material for detection of human exposure to PCDDs and PCDFs by cigarette smoking[3].
From above results, hair analysis seems to be one of useful means for monitoring human exposure to PCDDs, PCDFs and non-ortho chlorine substituted coplanar PCBs (Co-PCBs), because the hair is easily collected from people in wide ranges of age, resident area, eating habits and work. Therefore, we studied an analytical methods for PCDDs, PCDFs and Co-PCBs in hair[4]. Consequently, it was found the hair levels of PCDDs, PCDFs and Co-PCBs increased with a decrease of the hair cutting size, that is, the powdered sample was considered to be the most suitable for extraction with toluene under reflux. As a matter of fact, however, the powdering of hair sample was very difficult due to the hard cuticle of hair.
In this study, we confirmed that an alkaline decomposition method was effective for analysis of chlorinated pollutants in hair sample. The results are given in this paper.

2. EXPERIMENTAL

Sample

About 1 kg of human hair was collected from a hundred normal male persons at a barber shop in Hirakata, Osaka, in April, 1994. The specimen was cut into a length of ca. 5 mm by hair clippers and then stirred well up. The sample was used for investigation on analytical method of chlorinated pollutants. Another hair samples were obtained from two persons at a rate of about once a month.

Method

1) Examination on hair washing frequency with a commercial shampoo
After spiking of internal standards (five $^{13}C_{12}$-PCDDs and five $^{13}C_{12}$-PCDFs, each 500 pg;

Table.1 HPLC condition

Machine :	Shimadzu LC-6A
Column :	Sandon Hypercarb PCB (porous graphitic carbon) 50 mm (length) × 4.6 mm (i.d.) 7 μ m (particle diameter)
Injection vol. :	50 μ l
Detector :	UV-254 nm
Eluent :	Fraction 1 (n-hexane 0-3.5 ml) Chlordanes and PCBs except for mono-ortho and non-ortho substituted PCBs Fraction 2 (1% toluene/n-hexane 3.5-8.5 ml) Mono-ortho substituted PCBs Fraction 3 (50% toluene/n-hexane 8.5-15.5 ml) Non-ortho substituted PCBs Fraction 4 (toluene (60℃) reverse flow 15.5-23.5 ml) PCDDs and PCDFs

Table.2 Solubility of hair sample under various alkaline conditions

	1 hr	2 hr	3 hr	4 hr
3N KOH/EtOH	++	+++	+++	+++
2N KOH/EtOH	+	++	++(+)	++(+)
3N KOH/H_2O	+	++(+)	+++	+++
2N KOH/H_2O	+	++	++	+++
1N KOH/H_2O	-	++	++	++
3N NaOH/EtOH	++	++(+)	+++	+++
3N NaOH/H_2O	+	++	+++	+++
2N NaOH/H_2O	-	++	++(+)	++(+)

three $^{13}C_{12}$-Co-PCBs, each 1000 pg), unwashed, one time washed and two times washed hair samples (15 g) were respectively extracted with 200 ml of toluene for 3 hours 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 multi-layer column containing Na_2SO_4 (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 an eluent of n-hexane (170 ml). The eluate was concentrated to 5 ml and chromatographed into four fractions by HPLC with a porous graphitic carbon column under the conditions shown in Table 1.

After addition of keeper solvent (n-decane, 30 μl), the third and fourth eluates containing Co-PCBs and PCDDs/PCDFs, respectively, were concentrated and then adjusted to a volume of 10 μl with n-decane.

Above two purified extracts were analyzed on a 30 m J&W DB-5 for Co-PCBs and PCDDs/PCDFs in an electron impact-single ion monitoring mode at a resolution of 8000 using a

Hewlett Packard 5890J gas chromatograph-JEOL SX-102 mass spectrometer. The results were corrected for the recovery of $^{13}C_{12}$-labeled internal standards.

2) Examination on hair decomposition under various alkaline conditions

Hair sample (1 g) and alkaline solution (8 ml) were added into a test tube (10 ml), and then the test tube was mechanically shaken at room temperature. The conditions of alkaline solution and shaking time were given in Table 2. After shaking, the solubility of hair was checked.

3) Examination on stability of OCDD in alkaline solution

Ten μl of 1 ppm OCDD solution was poured into a test tube containing of 4 ml of each alkaline solution described in Fig. 3. The test tube was mechanically shaken during a period of 0.5 to 4 hrs. at room temperature. After shaking, the solution was extracted twice with 4 ml of n-hexane. The extract was washed, concentrated, dried, dissolved with 100μl of n-decane and analyzed for OCDD using a gas chromatograph with ^{63}Ni-ECD.

4)Examination on time course of PCDDs and PCDFs in hair samples from two persons

After spiking of internal standards, hair samples (ca. 7 g) from one person were decomposed in 100 ml of 2N KOH/H_2O solution for 4 hrs. 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 porous graphitic carbon column. Hair samples from another person were extracted with toluene of 60 ml for 3 hrs. under reflux, followed by multi-layer column chromatography and HPLC with a porous graphitic carbon column.

The purified extracts were analyzed for Co-PCBs, PCDDs and PCDFs using capillary column GC-MS as described above.

3. RESULTS AND DISCUSSION

In general, environmental chlorinated pollutants in hair are considered to be brought via body and via atmospheric deposition[2,3]. This is very important for an assessment of human exposure to them using hair sample as an indicator. Therefore, we tried to detect the exposure via the body.

As shown in Fig. 1, one time washing with common surfactant decreased remarkably the levels of PCDDs and PCDFs in hair sample. The decline rates of PCDDs and PCDFs were 50 and 64%, respectively. One more washing, however, gave no effect for the elimination of both chemicals. In addition, both washed samples contained similar compositions of PCDDs and PCDFs.

From these results, PCDDs and PCDFs on the hair surface deposited via atmospheric transfer are surmised to be completely removed by the first washing. The remainings are considered to be contained in the inner part of hair. In our earlier study[4], the extractable amounts of PCDDs, PCDFs and Co-PCBs with toluene under reflux were significantly higher in hair sample with 1 mm cutting size than 5 mm cutting size (Fig. 2). This indicates the pollutants present at the inside of hair are difficult to extract through the hard cuticle of hair. Therefore, we consider that the exposure via body is stable against common surfactant washing with less extractable ability than toluene reflux extraction.

Table 2 shows the solubility of hair under various conditions. Marks of -, +, ++, ++(+) and +++ mean the roughly soluble amount of 0, 10, 50, 90 and 100%, respectively. In the case

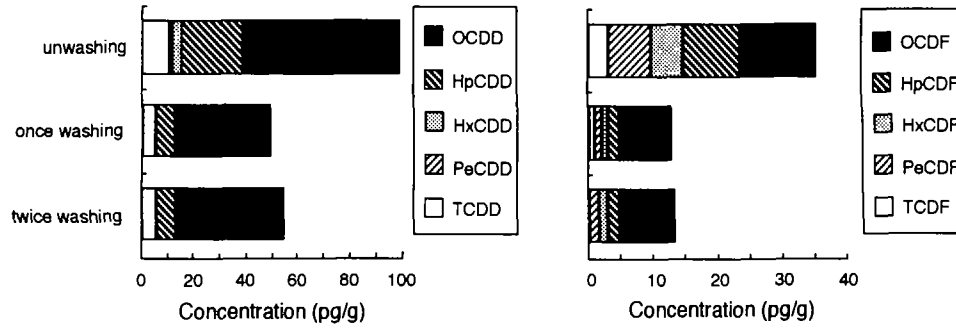

Fig.1 Effect on contamination levels of PCDDs and PCDFs in hair sample by washing with common surfactant

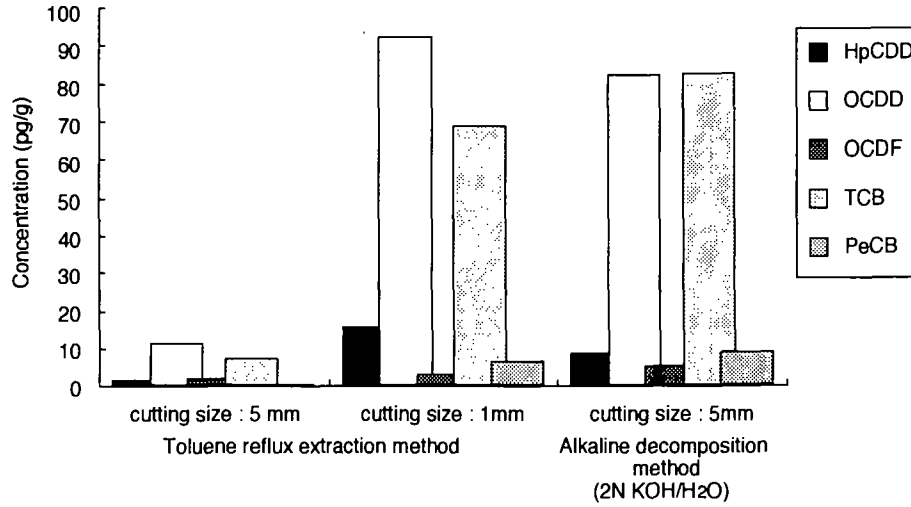

Fig.2 Comparison on the levels of major chlorinated pollutants in hair sample

of 2N alkaline solution, the whole hair sample dissolved in only KOH/H_2O by 4 hrs. mechanical shaking. However, the complete dissolution failed in 1N KOH/H_2O. On the other hand, all kinds of 3N alkaline solution decomposed entirely the sample during a shorter treatment time of 3 hrs.

PCB congeners were stable in hot alkaline solution. Higher chlorinated PCDD and PCDF congeners, however, were revealed to be easily decomposed by hot alkaline treatment[5]. Especially, the degradation speed was the greatest in OCDD among all congeners. Therefore, we tested the stability of OCDD by mechanical shaking with various alkaline solutions (Fig. 3).

As shown in Fig. 3, OCDD was stable in all four solutions under a half hour shaking. However, 2N KOH/EtOH decomposed ca. 30% of OCDD for 1 hr. In the case of more than 2 hrs. treatment, only 2N KOH/H_2O gave no adverse effect to OCDD. A magnitude of

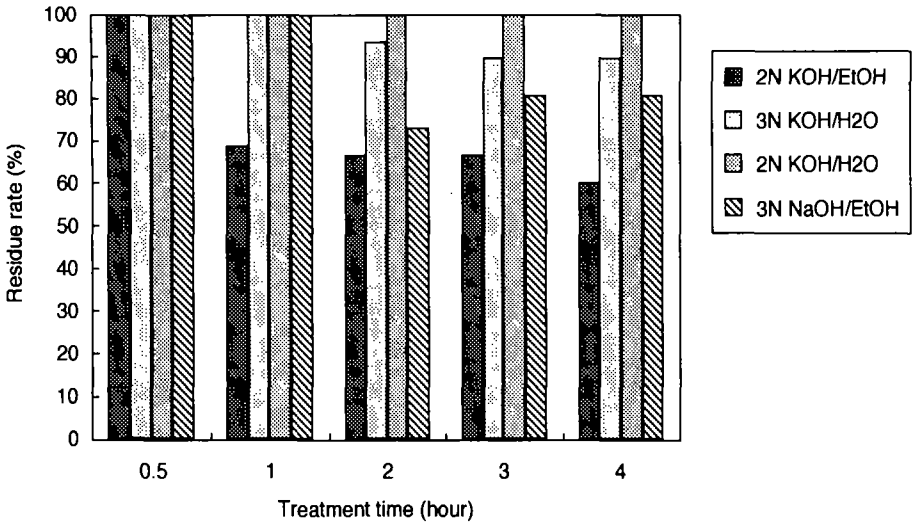

Fig.3 Decomposition of OCDD under various alkaline condition

Table.3 Time course of 2,3,7,8-PCDDs, 2,3,7,8-PCDFs and Co-PCBs in hair samples collected at three different periods from two same persons

Sample No.	Sampling Time	Concentration (pg/g)					
		HxCDD	HpCDD	OCDD	OCDF	TCB	PeCB
person 1							
# 1-1*	Apr.,1994	1.43	13.2	57.3	23.7	85.0	3.64
# -2	May.,1994	2.94	19.9	151	254	106	3.57
# -3	Jul.,1994	0.91	11.5	69.8	47.2	74.0	2.49
person 2							
# 2-1**	May.,1994	1.40	11.9	105	13.3	85.1	10.6
# -2	Jul.,1994	0.65	8.22	62.6	14.2	70.4	11.2
# -3	Sep.,1994	1.19	12.8	63.0	26.4	69.0	9.99

* : extracted with toluene under reflux

** : decomposed in 2N KOH solution and extracted with n-hexane

degradation ability was arranged in order of 2N KOH/EtOH > 3N NaOH/EtOH > 3N KOH/H_2O > 2N KOH/H_2O.

From results of hair solubility and OCDD degradation, we select 2N KOH/H_2O as a suitable solution for analysis of PCDDs, PCDFs and Co-PCBs in hair.

Table 3 shows concentrations of 2,3,7,8-PCDDs, 2,3,7,8-PCDFs and Co-PCBs in hair samples collected at three different periods from two same persons. Hair samples from

person 1 were analyzed using our old method including essentially once washing, 1 mm cutting in size, toluene extract, multi-layer column chromatography and HPLC clean-up. While our new method including once washing, 5 mm cutting in size, alkaline decomposition in 2N KOH/H_2O, multi-layer column and HPLC clean-up, was adapted to samples from person 2.

In general, the body burdens of PCDDs, PCDFs and Co-PCBs are surmised to be roughly constant during a period of a few months. In the case of our old method, however, there was a great difference in the level of each pollutant. Especially, the great discrepancy was seen in OCDD and OCDF. The main cause is speculated to be attributable to a difference in cutting size of hair. On the other hand, in our new method, each contaminant gave a roughly constant level in the time course of May to September, 1994.

From all results, it was revealed that human hair was an excellent indicator for human body burdens of PCDDs, PCDFs and Co-PCBs, and that our newly developed method was excellent for the determination of the chlorinated compounds in hair sample.

We will present the results of the exposure levels of PCDDs, PCDFs and Co-PCBs in hair samples from unexposed normal persons.

4. REFERENCES

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