INVESTIGATION ON ANALYSIS OF POLYCHLORINATED DIBENZO-P-DIOXINS, POLYCHLORINATED DIBENZOFURANS AND NON-ORTHO CHLORINE SUBSTITUTED COPLANAR PCBS IN HUMAN HAIR

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1. INTRODUCTION

In 1991, Ohgami et al. reported that human hair was a useful material as an indicator of human pollution by PCBs and polychlorinated quaterphenyls (PCQs)[1], that is, patients with Yusho disease concerning with ingestion of cooking rice bran oil contaminated with PCB product (Kanechlor) in our western country showed remarkable higher hair levels of PCBs (28.92 ppb) and PCQs (0.53 ppb) than did unexposed normal persons (2.43 and <0.1 ppb, respectively). Regarding toxic polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs), the human hair was 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].
Taking above things into consideration, 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 the wide ranges of year, resident area, eating habits and work. Therefore, this study was carried out to establish an analytical methods for PCDDs, PCDFs and Co-PCBs in hair in order to assess human exposure to them.

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 October, 1993. In this shop, the hair was washed with a commercial shampoo before haircutting. The specimen was cut into a length of ca. 5 mm by hair clippers and then stirred well up.

Method

1) Examination on a suitable solvent for extraction

After spiking of internal standards (five $^{13}C_{12}$-PCDDs and five $^{13}C_{12}$-PCDFs, each 500 pg; three $^{13}C_{12}$-Co-PCBs, each 1000 pg), four portions of hair sample (15 g) were respectively extracted with 200 ml of benzene, toluene, ethanol (EtOH), 95% ethanol-water/toluene (1:1) (EtOH/toluene) and 95% ethanol-water/benzene (1:1)(EtOH/benzene) 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 three fractions with successive eluents of 50 ml of 0.1% methylene chloride in n-hexane, 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). The third eluate containing PCDDs, PCDFs and Co-PCBs was concentrated to 3 ml. After addition of keeper solvent (n-decane, 30 μl), the concentrated eluate was left for complete evaporation of n-hexane in room temperature and then adjusted to a volume of 30 μl with n-decane.

Above finally purified extract was analyzed on a 30 m J&W DB-5 for Co-PCBs and for hepta- and octachlorinataed PCDDs and PCDFs, and on a 60 m Supelco 2331 for tetra- through hexachlorinated PCDDs and PCDFs in an electron impact-single ion monitoring mode at a resolution of 7000 using a Hewlett Packard 5890J gas chromatograph-JEOL SX-102 mass spectrometer. The result were corrected for the recovery of $^{13}C_{12}$-labeled internal standards.

2) Examination on hair length for extraction

Above the hair sample cutting with ca. 5 mm length was cut into smaller length of ca. ' mm by agitating with sea sand in a glass motor. Samples with ca. 5 mm and 1 mm were respectively analyzed for PCDDs, PCDFs and Co-PCBs according to above the method.

3-1) Examination on extraction time

After addition of $^{13}C_{12}$-labeled internal standards, sample with length of ca. 5 mm (45 g) was extracted with 600 ml of toluene. A portion of 80 ml was taken out at 1, 3, 5, 7, 9, 12 and 24 hrs., respectively after the extraction. Each extract was analyzed according to above the method.

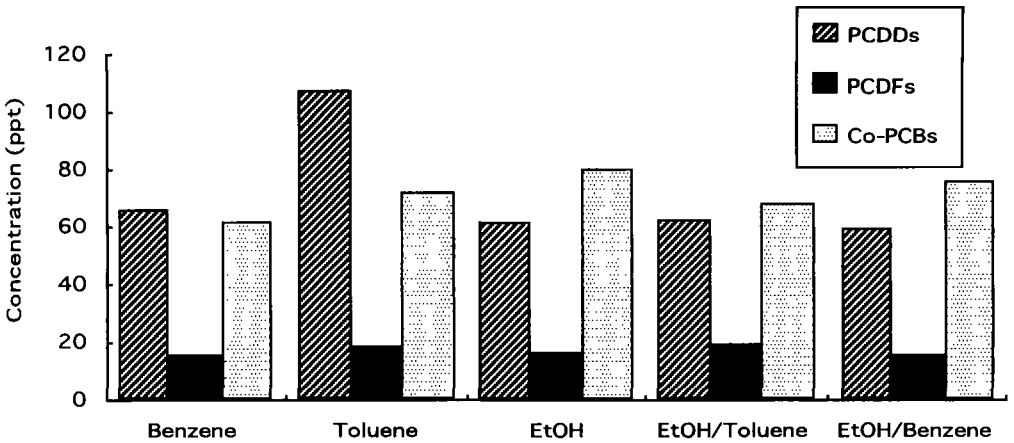
3-2) Examination on extraction time

After addition of $^{13}C_{12}$-labeled internal standards, the sample with length of ca. 5 mm (30 g) was extracted with 500 ml of toluene for 1 hour. After removing completly the extract, the same amounts of the internal standards and toluene were added into the original hair sample and extacted for two hours. The same trial was repeated more five times. Each extract was analyzed according to above the method.

3. RESULTS AND DISCUSSION

The human hair is considered to be polluted by PCDDs, PCDDs and Co-PCBs via two routes of blood stream and air. Schramm et a;.[2] revealed that washing with common surfactant was very effective to remove PCDDs and PCDFs on the surface of hair, both of which had been brought through air. This indicates the washed hair contains pollutants brought via

Fig. 1. Concentrations of 2,3,7,8-PCDDs, 2,3,7,8-PCDFs and Co-PCBs extracted from a sample of human hair with 5mm length with various solvents



blood stream. Therefore, in this study, human hair samples washed with a surfactant were used in order to establish an analytical method of PCDDs, PCDFs and Co-PCBs.

Figure 1 shows the concentrations of 2,3,7,8-chlorine substituted PCDDs (2,3,7,8-PCDDs), 2,3,7,8-chlorine substituted PCDFs (2,3,7,8-PCDFs) and Co-PCBs in the hair sample extracted with five solvents. In general, toluene and 95% ethanol-water were respectively reported to be the most suitable solvent for extraction of PCDDs and PCDFs from samples of sediment and soil[4] and from paper and pulp[5]. Therefore, five solvents in Table 1 were investigated for the extraction efficiency of PCDDs, PCDFs and Co-PCBs from hair sample.

As shown in Fig. 1, the sum of PCDDs, PCDFs and Co-PCBs was arranged in order of toluene (199 ppt), EtOH (159 ppt), EtOH/benzene (151 ppt), EtOH/toluene (150 ppt) and benzene (142 ppt) in a magnitude. Especially, toluene gave the highest extract efficiency for PCDDs, showing the level (108 ppt) to be about 2 times greater than those (60 to 65 ppt) of other solvents. In a case of PCDFs, however, EtOH/toluene was the most effective solvent. In the meantime, EtOH was the most excellent solvent for Co-PCBs.

As described above, the best solvent varied on the kind of compound. Taking the sum of all compounds into consideration, toluene seems to be the best as an extraction solvent for them from hair.

Figure 2 illustrates the ratios of 1,2,3,4,6,7,8-HpCDD and OCDD extracted from hair samples with 1 mm length versus 5 mm length. In all five extraction solvents, the shorter sample gave higher concentrations with the ratio of 4.4 to 11 times greater in HpCDD than did the longer one. The former sample showed also a lifted ratio (6.7 to 8.2) for OCDD in all reagents except EtOH. This indicates that the shorter the hair length is, the higher the extraction efficiency for HpCDD and OCDD from hair becomes. In actuality, however, it is very difficult to prepare the hair sample with length of less than 1 mm. Therefore, we decided to use hair with the size of ca. 1 mm for monitoring of human pollution by chlorinated compounds.

In the first trial for determination of extraction time, a constant volume of toluene extract was taken out at 1, 2, 5, 7, 9, 12 and 24 hours, respectively after the experiment start.

Fig. 2. Ratios of 1,2,3,4,6,7,8-HpCDD and OCDD extracted from a sample of human hair with 1 mm length versus 5 mm length with various solvents

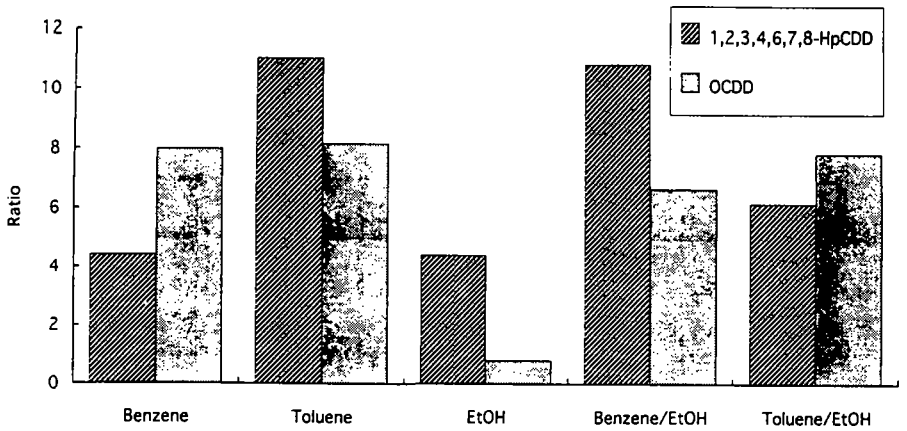
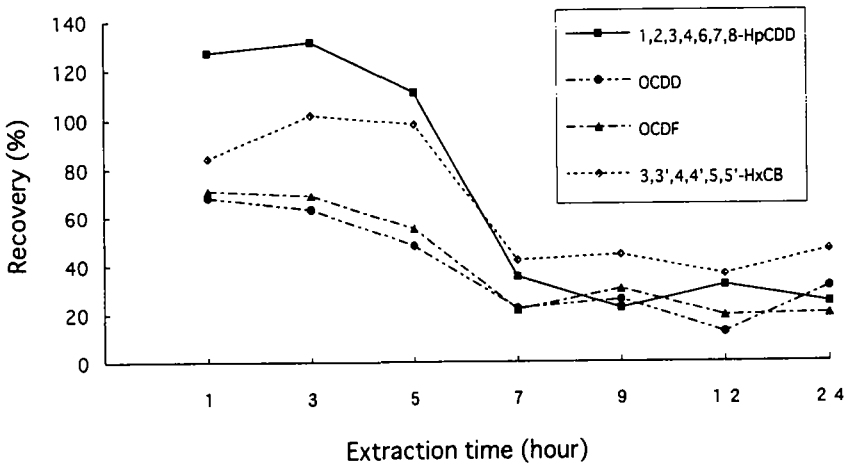


Fig. 3. Time course of recovery of $^{13}C_{12}$-labeled internal PCDD, PCDF and Co-PCB isomers



Blelieve it or not, the recoveries of all $^{13}C_{12}$-labeled internal standards had a dectrase tendency in a time course of the extraction, showing the declin to be remarkable following the 5 hours. For example, the recoveries of OCDD at the period of 1, 3, 5, 7, 9, 12 and 24 hours were 68, 63, 48, 22, 26, 12 and 30%, respectively (Fig. 3). On the other hand, the analytical value of native OCDD increased with increasing of extraction time, showing the levels at 1, 3, 5, 7, 9, 12 and 24 hours to be 75, 82, 97, 130, 144, 195 and 196 ppt, respectively. In contrast with this, those of other native PCDDs, PCDFs and Co-PCBs gave a decrease tendency in the time course of extraction. In the mean time, sampling of the toluene extract resulted into an increase of the ratio of hair weight to solvent volume in the extraction flask.

These results gave us a suggestion that added internal standards and extracted native compounds might be absorbed at their independent adsorption rates on the surface of hair and that the rate might be different between the internals and their corresponding natives. Therefore, as above described in the term [3-2)] in "Method", another trial was carried out

Table 1. Amounts of PCDD, PCDF and Co-PCB isomers extracted from a sample of human hair during a period of each extraction time with toluene under reflux

Extraction Time (hr)	Concentration (pg/g)					
	HpCDD	OCDD	HpCDF	OCDF	TCB	PeCB
0~1	18	290	4.5	1.0	240	25
1~3	1.5	11	0.35	<0.2	18	1.5
3~5	<0.2	<1.8	<0.01	<0.2	<3.9	<0.1
5~7	<0.2	<1.8	<0.01	<0.2	<3.9	<0.1
7~9	<0.2	<1.8	<0.01	<0.2	<3.9	<0.1
9~12	<0.2	<1.8	<0.01	<0.2	<3.9	<0.1
12~24	<0.2	<1.8	<0.01	<0.2	<3.9	<0.1

HpCDD: 1,2,3,4,6,7,8-HpCDD, HpCDF: 1,2,3,4,6,7,8-HpCDF, TCB: 3,3',4,4'-TCB, PeCB: 3,3'4,4',5-PeCB

for the determination of suitable extraction time. Table 1 shows amounts of PCDDs, PCDFs and Co-PCBs extracted from a hair sample during the period of each extraction time with toluene under reflux. From this result, it was revealed that a great part of extractive amounts of all compounds was derived from the duration of the first 1 hour 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, of all extractive amounts of them. In the meantime, there was no observation on extractive amounts of all compounds in a period of 3 to 5 hours. Consequently, the suitable extraction time was concluded to be 3 hours. We will present the results of the most suitable extraction solvent volume for hair sample and the concentrations of PCDDs, PCDFs and Co-PCBs in hair from unexposed normal persons.

4. REFERENCES

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