Concentrations of Polychlorinated Biphenyls in Blood Collected from Yusho Patients during Medical Check-ups Performed from 2004 to 2007

Todaka, Takashi
Department of Dermatology, Graduate School of Medical Sciences, Kyusyu University

Hori, Tsuguhide
Fukuoka Institute of Health and Environmental Sciences

Yasutake, Daisuke
Fukuoka Institute of Health and Environmental Sciences

Yoshitomi, Hideaki
Fukuoka Institute of Health and Environmental Sciences

他

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Takashi TODAKA1, Tsuguhide Hori2, Daisuke YASUTAKE2, Hideaki YOSHITOMI2, Hironori HIRAKAWA2, Daisuke OGOZUKA2, Jumboku KAJIWARA2, Takao IIDA3, Takesumi YOSHIMURA2 and Masutaka FURUE1,4

1) Department of Dermatology, Graduate School of Medical Sciences, Kyusyu University, Fukuoka, Japan
2) Fukuoka Institute of Health and Environmental Sciences, Fukuoka, Japan
3) Kitakyushu Life Science Center, Fukuoka, Japan
4) Research and Clinical Center for Yusho and Dioxin, Kyushu University Hospital, Fukuoka, Japan

Abstract To elucidate the exposure levels of polychlorinated biphenyls (PCBs) for Yusho patients, we conducted a congener–specific analysis of polychlorinated biphenyls (PCBs) in blood collected from 242, 237, 300, and 96 Yusho patients during medical check-ups performed from 2004 to 2007, respectively, and in samples from 74, 113, 125, and 148 Yusho–suspected persons during those same years, respectively, and compared the individual congener concentrations of PCBs among the groups of Yusho patients, Yusho–suspected persons, and normal controls with the concentrations previously reported. Among the 209 PCB congeners, 8 congeners of mono–ortho PCBs and 56 congeners of non–dioxin–like PCBs were identified in the blood of Yusho patients and Yusho–suspected persons. Among the PCB congeners measured in the present study, hexaCB–153, hexaCB–138, heptaCB–180, and heptaCB–182/heptaCB–187 showed high ratios to total concentrations of 64 PCB congeners detected in the blood of Yusho patients and Yusho–suspected persons from 2004 to 2007, and the profiles of the major congeners were the same as those obtained in normal controls. With respect to the minor congeners of PCBs, several differences were observed among the three groups. The sums of the concentrations of 64 PCB congeners in the blood of Yusho patients from 2004 to 2007 were 645, 760, 667, and 510 ng g⁻¹ lipid for each year, respectively, and the concentrations were 1.5, 1.8, 1.5, and 1.2 times higher than those of normal controls for each year, respectively. Those of the Yusho–suspected persons were approximately 0.8, 1.1, 0.9, and 1.0 times higher than those of normal controls for each year, respectively. The ratios of heptachlorinated biphenyls (heptaCBs) to the total concentrations of 64 PCB congeners in the blood of Yusho patients and Yusho–suspected persons from 2004 to 2007 tended to be slightly higher than those in the normal controls. From the results comparing the concentrations of 64 PCB congeners in the blood between Yusho patients and normal controls, the concentrations of hexaCB–156, hexaCB–157, heptaCB–181, and heptaCB–189 for Yusho patients were 3.4, 3.8, 3.9, and 3.8 times, respectively, 3.9, 4.1, 3.9, and 4.4 times, respectively, 3.6, 3.9, 5.0, and 4.1 times, respectively, and 2.3, 2.5, 2.7, and 2.9 times higher than those of the normal controls for each year from 2004 to 2007, respectively. These results indicated that Yusho patients still have higher concentrations of hexaCB–156, hexaCB–157, heptaCB–181, and heptaCB–189 in their blood than do unaffected people, even though over 35 years have passed since the outbreak of Yusho. These four congeners can therefore be considered to be the most important congeners for evaluating the PCBs exposure of Yusho patients.

Corresponding author: Takashi TODAKA
Tel.: + 81-92-921-9946; Fax.: + 81-92-928-1203
E-mail address: todaka@fihes.pref.fukuoka.jp
Introduction

The Yusho poisoning accident, which affected over 1800 people, occurred in 1968 in western Japan, and was caused by the ingestion of rice bran oil that contained the following contaminants: PCBs, polychlorinated dibenzofurans (PCDFs), polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated quarterphenyls, and polychlorinated terphenyls. Since the Yusho outbreak, medical care services and health examinations of the subjects have been carried out by the Yusho study group investigating this disease. From the results of extensive research by the Yusho study group, PCDFs were concluded to be the primary cause of the Yusho disease symptoms. In order to support the health care of Yusho patients, we measured the concentrations of PCDDs, PCDFs, and non-ortho PCBs in blood collected from 279, 269, 242, 237, 300, and 96 Yusho patients during medical check-ups performed from 2002 to 2007, respectively, and in samples from 92, 74, 74, 114, 125, and 148 Yusho-suspected persons during those same years, respectively. We also measured the concentrations of these dioxin-like compounds in the blood of 127 normal controls unaffected by Yusho whose ages were similar to those of the Yusho patients. The results showed that PCDFs in the blood of Yusho patients, in particular 2,3,4,7,8-pentachlorinated dibenzofuran (2,3,4,7,8-pentaCDF), are still present at much higher concentrations than in the blood of unaffected people.

Over 35 years have passed since the Yusho outbreak, which occurred because Yusho patients had ingested rice oil contaminated with large amounts of PCBs that were used as a heat-transfer medium in the process of rice oil production. Survey studies of the concentrations of PCB congeners in the blood of Yusho patients are very important when considering the health status of these patients. With respect to the analysis of PCB congeners in the blood of Yusho patients, exposure studies of total PCB levels and the major congeners in the blood were primarily developed beginning 5 years after the outbreak of Yusho. However, a survey study regarding the full congener-specific concentrations of PCBs in the blood of Yusho patients have not been conducted. Recently, advances in the analytic methods used for quantification of PCB congeners have made it much easier to evaluate the exposure levels of PCBs in human. We previously conducted a congener-specific analysis of PCBs in the blood collected from 15 Yusho patients and 43 Yusho-suspected persons in 2006 for a preliminary study. We also measured the 64 PCB congener concentrations in the blood of 127 normal controls unaffected by Yusho. Although our studies have led to reports of the congener concentrates of PCBs in the blood of Yusho patients and Yusho-suspected persons, the studies have suffered from small sample size. To obtain the most useful data for evaluating the exposure levels of PCB congeners in the blood of Yusho patients, it is necessary to measure the concentrations of full PCB congeners in the blood collected from many more Yusho patients. Consequently, the data from congener profiles regarding PCBs in the blood of Yusho patients may provide us with newly important information related to exposure evaluation of patients and with valuable information for future epidemiologic studies.

In this study, we carried out a congener-specific analysis of PCBs in the blood collected from 242, 237, 300, and 96 Yusho patients during medical check-ups performed from 2004 to 2007, respectively, and in samples from 74, 113, 125, and 148 Yusho-suspected persons during those same years, respectively, and compared with the concentrations of PCB congeners among the groups of Yusho patients, Yusho-suspected persons, and normal controls that had been previously reported.
Materials and Methods

1. Sampling

Medical check-ups for Yusho patients have been conducted annually to determine the health status of patients since the outbreak of the Yusho incident. The medical check-ups are available not only to those persons officially registered as Yusho patients but also to Yusho-suspected persons who regard themselves as potential victims. Both officially registered Yusho patients and Yusho-suspected persons are examined based on the "Diagnostic Criteria for Yusho". The blood samples examined in this study were collected from 316, 351, 425, and 244 participants who received a medical check-up for each year from 2004 to 2007, respectively, from whom informed consent was obtained. The 316 participants included 242 Yusho patients (mean : 65.5 years) and 74 Yusho-suspected persons (mean : 54.7 years) in 2004, the 351 participants included 237 Yusho patients (mean : 67.3 years) and 114 Yusho-suspected persons (mean : 54.7 years) in 2005, the 425 participants included 300 Yusho patients (mean : 66.3 years) and 125 Yusho-suspected persons (mean : 50.7 years) in 2006, and the 244 participants included 96 Yusho patients (mean : 57.8 years) and 148 Yusho-suspected persons (mean : 56.5 years) in 2007. Among 114 Yusho-suspected persons in 2005, the concentrations of PCB congeners in the blood of 113 persons (mean : 56.8 years) were measured in the present study. Blood samples of 10 ml were collected using a vacuum blood-collecting tube containing heparin and were stored at 4°C until analyses for congener concentrations of PCBs.

2. Materials

Native congeners of mono-ortho PCBs and non-dioxin-like PCBs were purchased from Wellington Laboratories (Guelph, Canada). [13C12] -congeners of mono-ortho PCBs and non-dioxin-like PCBs as internal standards were also purchased from Wellington Laboratories. An active carbon column was prepared as follows: active carbon was purchased from Nacalai Tesque (Kyoto, Japan), refluxed 3 times with toluene for 1 hour, and dried in vacuum, after which 500 mg of the active carbon was mixed with 500 g of anhydrous sodium sulfate (Wako Pure Chemical Industries, Ltd., Tokyo, Japan). A silver nitrate/silica gel was purchased from Wako Pure Chemical Industries, Ltd. An active carbon-dispersed silicagel was purchased from Kanto Chemical Industries, Ltd., Tokyo, Japan. All reagents and solvents used in this experiment were of the analytic grade of dioxin that is commercially available. All glassware instruments used in this experiment were treated in a high-temperature oven (ALP Co. Ltd., Tokyo, Japan) at 450°C for 6 hours.

3. Analysis of non-dioxin-like PCBs

The extraction and purification of PCB congeners from the blood samples was performed using a previously reported method. Congener-specific analysis of PCBs was measured using a high resolution gas chromatography/high resolution mass spectrometry (HRGC / HRMS).

4. Quality control

The limit of detection (LOD) for each congener of PCBs was determined at a signal-to-noise ratio of three on the chromatogram of a standard sample. The limit of quantification for each congener of PCBs was assessed at 0.03 pg g⁻¹. To evaluate the accuracy and reliability in the congener-specific analysis of PCBs, our laboratory in 2007 prepared human blood samples for quality control and attempted to carry out a quality control study for the analysis of these PCB congeners in human blood. Measurements of 64 PCB congeners that were measured in the present study among 209 PCB congeners requested from three different analysis organizations and their results were compared with our results. It was confirmed that the results
obtained by our laboratories were almost identical to those obtained by the three different analysis organizations. The average variation among the total levels of these PCB congeners in human blood samples obtained by four laboratories was within 15% and was considered an acceptable difference. In addition, our laboratory’s analytical method for PCB congeners demonstrated high reproducibility based on experiments conducted using the same control blood sample for ten weeks. These findings indicate that our laboratory’s analytical method for PCB congeners in human blood provides correct results.

**Results and discussion**

PCBs form a family of 209 congeners differing in number (mono, di, tri, tetra, penta, hexa, hepta, octa, nona, and deca) and position (2, 2’, 3, 3’, 4, 4’, 5, 5’, 6, and 6’) of the chlorine atoms on the two basic benzene rings. The 209 PCB congeners consist of 12 dioxin–like PCBs (non-ortho PCBs and mono-ortho PCBs) and 197 non–dioxin–like PCBs. Among the 209 PCB congeners, 8 congeners of mono-ortho PCBs and 56 congeners of non-dioxin–like PCBs were identified in the blood of Yusho patients and Yusho–suspected persons. The concentrations of PCB congeners in the blood of Yusho patients and Yusho–suspected persons from 2004 to 2007, including the dates of the normal controls that had been previously reported, are presented in Table 1–2. We compared the congener patterns of PCBs in normal controls of our studies with those from 24 healthy Japanese volunteers (12 men and 12 women; age range 25–46 years) that had previously been reported in Japan. Among 93 PCB congeners that were measured in the blood, as previously reported, 63 were commonly detected in the blood of normal controls in the present study. The total concentrations of the 63 congeners contributed approximately 96% of the total concentrations of 93 PCB congeners. These 63 PCB congeners measured in the present study may be considered to be the predominant PCB congeners in humans.

Among the 64 PCB congeners that were measured in the present study, hexaCB–138, hexaCB–153, heptaCB–180, and heptaCB–182/heptaCB–187 showed high ratios to the total concentrations of 64 PCB congeners detected in the blood of Yusho patients, Yusho–suspected persons, and normal controls (Table 1–2). Other PCB congeners contributed less than 6% of the total concentrations of these PCB congeners. Although the patterns of the major PCB congeners in the blood of Yusho patients and Yusho–suspected persons from 2004 to 2007 were almost the same as those obtained in normal controls, several differences with respect to the minor PCB congeners were observed among the three groups.

The sums of the concentrations of 64 PCB congeners in the blood of Yusho patients from 2004 to 2007 were 40–3032 (mean : 645, median : 536) ng g⁻¹ lipid in 2004, 40–4723 (mean : 760, median : 575) ng g⁻¹ lipid in 2005, 74–2432 (mean : 667, median : 553) ng g⁻¹ lipid in 2006, and 51–2252 (mean : 510, median : 357) ng g⁻¹ lipid in 2007, respectively, and the concentrations were 1.5, 1.8, 1.5, and 1.2 times higher than those of normal controls for each year, respectively (Table 1). In the case of Yusho–suspected persons, the concentrations were 20–1418 (mean : 355, median : 317) ng g⁻¹ lipid in 2004, 64–4055 (mean : 490, median : 351) ng g⁻¹ lipid in 2005, 18–1850 (mean : 397, median : 257) ng g⁻¹ lipid in 2006, and 19–2183 (mean : 440, median : 293) ng g⁻¹ lipid in 2007, respectively, which were almost the same or slightly lower than those of normal controls (Table 2).

The arithmetic mean concentrations of triCBs, tetraCBs, pentaCBs, hexaCBs, heptaCBs, octaCBs, nonaCBs, and decaCB–209 in the blood of Yusho patients were 1.9, 16, 45, 292, 228, 57, 4.5, and 1.4 ng g⁻¹ lipid in 2004, respectively, 2.5, 21, 53, 331, 268, 785.0, and 1.6 ng g⁻¹ lipid in 2005, respectively, 1.7, 19, 49, 305, 240, 46, 5.3, and 1.5 ng g⁻¹ lipid in 2006, respectively, and 1.6, 13, 36, 227, 188, 38, 4.4, and 1.4

(51)
Table 2: Concentrations of PCB congeners from 2004 to 2007

<table>
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<th>PCB Type</th>
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<th>2006</th>
<th>2007</th>
<th>Control</th>
<th>Note</th>
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<td>1443</td>
<td>16638</td>
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</table>

Note: The p-values for the medical check-up for Yusho-suspected persons from 2004 to 2007 were 0.35, 0.42, and 0.34 for each year, respectively.

ng g⁻¹ lipid in 2007, respectively (Table 1). In the case of Yusho-suspected persons, these concentrations were 1.9, 17, 36, 154, 115, 28, 2.4, and 1.0 ng g⁻¹ lipid in 2004, respectively, 3.4, 26, 46, 195, 164, 50, 3.3, and 1.4 ng g⁻¹ lipid in 2005, respectively, 1.7, 19, 39, 170, 138, 26, 3.0, and 1.1 ng g⁻¹ lipid in 2006, respectively, and 2.0, 19, 41, 185, 157, 31, 3.4, and 1.2 ng g⁻¹ lipid in 2007, respectively (Table 2). On the other hand, these concentrations in the blood of normal controls were 2.6, 26, 51, 188, 128, 32, 3.1, and 1.4 ng g⁻¹ lipid, respectively. The concentrations of hexaCBs, heptaCBs, octaCBs, and nonaCBs in the blood of Yusho patients were slightly higher than those of the normal controls.

The relative contribution ratios of the concentrations of triCBs, tetraCBs, pentaCBs, hexaCBs, heptaCBs, octaCBs, nonaCBs, and decaCB-209 to the total concentrations of the 64 PCB congeners compared the ratios among the groups of Yusho patients, Yusho-suspected persons, and normal controls. The ratios of the mono-ortho PCBs concentrations to the total concentrations of 64 PCB congeners for Yusho patients and Yusho-suspected persons were almost the same as those of normal controls. The ratios of the triCBs, tetraCBs, pentaCBs, hexaCBs, heptaCBs, octaCBs, nonaCBs, and decaCB-209 concentrations to the total concentrations of 64 PCB congeners for normal controls were 0.6, 6.0, 11.8, 43.5, 29.6, 7.4, 0.7, and 0.3%, respectively. In the case of Yusho-suspected persons, the contribution ratios of these eight concentrations were 0.5, 4.7, 10.0, 43.3, 32.5, 8.0, 0.7, and 0.3% in 2004, respectively, 0.7, 5.4, 9.5, 39.8, 33.5, 10.2, 0.7, and 0.3% in 2005, respectively, 0.4, 4.7, 9.8, 42.8, 34.7, 6.5, 0.8, and 0.3% in 2006, respectively, and 0.5, 4.3, 9.4, 42.1, 35.7, 7.0, 0.8, and 0.3% in 2007, respectively (Table 2). Finally, the ratios of these concentrations in Yusho patients were 0.3, 25, 7.0, 45.2, 35.3, 8.8, 0.7, and 0.2% in 2004, respectively, 0.3, 27, 7.0, 43.6, 35.2, 10.3, 0.7, and 0.2% in 2005, respectively, 0.3, 28.7, 7.4, 45.6, 36.0, 6.8, 0.8, and 0.2% in 2006, respectively, and 0.3, 25, 7.2, 44.5, 36.9, 7.5, 0.9, and 0.3% in 2007, respectively (Table 1); it was confirmed that the ratios of triCBs, tetraCBs, and pentaCBs to the total concentrations of the 64 PCB congeners for Yusho patients and Yusho-suspected persons were slightly lower than those of the normal controls, and the ratios of hexaCBs, octaCBs, nonaCBs, and decaCB-209 concentrations to the total PCB concentrations for Yusho patients and Yusho-suspected persons were nearly the same as those of the normal controls. However, the ratios of the concentrations of heptaCBs to the total concentrations of 64 PCB congeners in the blood for Yusho patients and Yusho-suspected persons tended to be slightly higher compared to those of the normal controls.

Among the PCB congeners measured in the present study, the concentrations of 64 PCB congeners in the blood for Yusho patients and Yusho-suspected persons were compared with those of the normal controls. The concentrations of hexaCB-156, hexaCB-157, heptaCB-181, and heptaCB-189 in the blood samples for Yusho patients were 27, 7.6, 0.3, and 3.9 ng g⁻¹ lipid in 2004, respectively, 31, 8.2, 0.3, and 4.6 ng g⁻¹ lipid in 2005, respectively, 29, 7.8, 0.4, and 4.2 ng g⁻¹ lipid in 2006, respectively, and 18, 5.0, 0.2, and 3.0 ng g⁻¹ lipid in 2007, respectively. These levels were 3.4, 3.8, 3.9, and 3.8 times, respectively, 3.9, 4.1, 3.9, and 4.4 times, respectively, 3.6, 3.9, 5.0, and 4.1 times, respectively, and 2.3, 2.5, 2.7, and 2.9 times higher than those of the normal controls for each year from 2004 to 2007, respectively (Table 1). In the case of Yusho-suspected persons, the concentrations were 0.7, 1.1, 0.8, and 0.8 times, respectively, 1.1, 1.1, 0.8, and 1.4 times, respectively, 0.9, 0.9, 2.1, and 1.1 times, respectively, and 1.0, 1.0, 1.1, and 1.2 times higher than those of the normal controls for each year from 2004 to 2007, respectively (Table 2). These findings indicated that Yusho patients still have higher concentrations of hexaCB-156, hexaCB-157, heptaCB-181, and heptaCB-189 in their blood than do unaffected persons more than 35 years after the Yusho incident. These four congeners can be considered the characteristic
congeners of PCBs in the blood of Yusho patients.

We have previously reported that the concentrations of 2,3,4,7,8-pentaCDF, which were the highest among the PCDF congeners for Yusho patients, were approximately 10 times higher than those of the normal controls. From the results obtained by the present study, the concentrations of hexaCB–156, hexaCB–157, heptaCB–181, and heptaCB–189, which were the highest among the PCB congeners in the blood samples for Yusho patients, were 3.4, 3.8, 3.9, and 3.8 times, respectively, 3.9, 4.1, 3.9, and 4.4 times, respectively, 3.6, 3.9, 5.0, and 4.1 times, respectively, and 2.3, 2.5, 2.7, and 2.9 times higher than those of the normal controls for each year from 2004 to 2007, respectively. These finding indicate that the ratios of 2,3,4,7,8-pentaCDF in the blood of Yusho patients for the more than 35 years since the outbreak of Yusho remain higher than those of PCB congeners.

In conclusion, the exposure levels of PCB congeners for Yusho patients and Yusho-suspected persons were able to be determined in the present study. The results indicated that Yusho patients still have higher concentrations of hexaCB–156, hexaCB–157, heptaCB–181, and heptaCB–189 in their blood than do unaffected people more than 35 years after the Yusho incident. These four congeners can be considered the most important congeners for evaluating the PCBs exposure of Yusho patients. We provide newly important information regarding the exposure evaluation of PCB congeners for Yusho patients and hope that these data can be used in future epidemiological investigations of Yusho patients.

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平成16年から19年度におけるカネミ油症検診者の血液中ポリ塩化ビフェニール濃度

1) 九州大学大学院医学研究院 皮膚科学分野
2) 福岡県保健環境研究所
3) 北九州生活科学センター
4) 九州大学病院 油症ダイオキシン研究診療センター

戸高 尊1), 堀 就英2), 安武 大輔2), 吉富 秀亮2), 平川 博仙2), 小野塚 大介2), 梶原 淳睦2), 飯田 隆雄3), 吉村 健清2), 古江 増隆1)4)

平成16年から平成19年度に、それぞれ242, 237, 300および96名の油症患者およびそれぞれ74, 113, 125および148名の未認定者から採取した血液中PCBの異性体濃度を測定し、一般健常人の結果と比較した。油症患者と未認定者の血液中において、PCB 64異性体を分離・定量した。油症患者の血液中、hexaCB-153, hexaCB-138, heptaCB-180およびheptaCB-182/187は他と比較して高い濃度を示したが、この結果は一般健常人と同様であった。平成16年から19年度に受診した油症患者血液中PCBの64異性体総濃度は、それぞれ645, 760, 667および510ng g⁻¹ lipidで、その濃度は一般健常人のそれぞれ1.5, 1.8, 1.5および1.2倍であった。未認定者の場合、総PCB濃度はほぼ健常人と同レベルであった。PCB総濃度に対する各異性体の割合を比較した結果、油症患者と未認定者のheptaCBs濃度は一般健常人より高かった。油症患者の血液中64PCB異性体濃度を健常人と比較した場合、hexaCB-156, hexaCB-157, heptaCB-181およびheptaCB-189の濃度は、一般健常人のそれぞれ2.3-3.9, 2.5-4.1, 2.7-5.0および2.9-4.4倍高い値を示した。以上の結果から、油症発症から35年が経過した現在も患者の血液中PCB濃度、特にhexaCB-156, hexaCB-157, heptaCB-181およびheptaCB-189濃度は以前高いレベルにあり、油症患者のPCB暴露を評価する上で最も重要な異性体であると言える。