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Monthly Variation in Blood Dioxin Level, Characteristics of Isomer Composition, and Isomer Changes in Residents near an Incineration Facility

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The degree of dioxin exposure in humans has been evaluated by assaying the dioxin level in biological specimens (Scumacher et al. 1999; Schecter 1998; Schecter et al. 1997). For biological specimens, blood, breast milk, fat, and organs such as the liver and adipose tissue are used, but only blood can be collected from wide populations of age groups regardless of gender. Many surveys have revealed contamination in humans using the blood dioxin concentration as the indicator. Based on previous reports, the control blood dioxin level was determined to be about 50 pg or lower (Schecter et al. 1997; Ryan et al. 1997; Päpke et al. 1997; Gonzalez et al. 1998), and these results allowed evaluation of the degree of human exposure based on the blood level. Some previous studies reported a high exposure level of up to several hundred pg in blood. However, to determine human exposure from the blood level, it is necessary to investigate in detail daily changes and changes due to intermittent exposure to the emission source in the blood level. We have already reported the change of blood level during a three-month period in the general population in whom the blood concentration was at the control level, and confirmed that the change of concentration was maintained at a tolerance level (Aozasa et al. 2001). In this study, to clarify monthly variation in higher dioxin level of the blood in residents near an incineration facility, the major source of dioxin, who are intermittently exposed, and the characteristics of isomer composition and its monthly changes, we researched the blood level in 13 residents near an incineration facility and evaluated the contamination level. In residents with higher dioxin levels, the blood level was monitored once a month and followed up for seven months.

MATERIALS AND METHODS

About 100 mL of blood was collected from 13 residents near an incineration facility between October 2000 and February 2001. The 13 residents consisted of seven males and six females and ages ranged from 60 to 80 years old shown in Table 1. Blood samples were kept standing overnight and centrifuged (3,000 rpm, 30 minutes), and the serum were collected. The serum, 25 g, was analyzed for 7 isomers of 2,3,7,8-PCDDs, 10 of 2,3,7,8-PCDFs 4 of non-, and 8 of mono-ortho Co-PCBs. The analysis procedure was described following.

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Table 1. Sex and age of subjects

Resident	Sex	Age
A	male	70
B	female	60
C	male	71
D	female	66
E	female	64
F	female	65
G	male	74
H	female	64
I	male	76
J	female	67
K	male	69
L	male	80
M	male	72

Twenty five grams of the serum was spiked with a mixture of ^{13}C-labeled internal standards for all 17 2,3,7,8-isomers and 12 Co-PCBs. The sample was prepared by mechanical shaking at room temperature for 2 hours with 2N KOH solution. The solution was extracted 2 times with 30 mL of hexane. The hexane phase concentrated to 10 mL was passed through a column containing 1 g of silver nitrate-coated silica gel ($AgNO_3$/silica) and eluted with 10 mL of hexane. The eluted solution was loaded on to an active carbon dispersed silica gel column (0.1 g), and separated into two fractions. The first fraction containing mono-ortho Co-PCBs was eluted with 5 mL of hexane and 4 mL of 25% methylene chloride in hexane. PCDDs, PCDFs and non-ortho Co-PCBs were eluted with 30 mL of toluene as the second fraction. These eluates to be spiked with 50 pg of ^{13}C-labeled recovery standards were respectively concentrated to 10 μL of n-nonane. The recovery standards used were ^{13}C-labeled (^{13}C-) 1,2,3,4-TCDD, ^{13}C-1,2,3,4,6,7,8-HxCDD, ^{13}C-2,3',4',5-TCB, ^{13}C-2,3',3,5,5'-PeCB, ^{13}C-2,2',3,4,4',5'-HxCB and ^{13}C-2,2',3,3',5,5',6-HpCB. Determinations were performed with the HP 6890 gas chromatograph and JEOL JMS 700M mass spectrometer at a resolution of 10000 in the EI-SIM mode. The concentration was calculated to the toxic equivalent (TEQ) using the WHO-TEF values (Van den Berg et al. 1998). The lipid concentration was determined gravimetrically using 10 g of serum, expressed as pg TEQ/g lipid.

Furthermore, blood samples were collected from these three subjects with higher dioxin level once a month for seven months from July 2001 to January 2002 and the dioxin level was measured in order to clarify variation in higher dioxin level, the characteristics of isomer composition and its changes.

RESULTS AND DISCUSSION

The aim of this study was to clarify monthly variation of the blood dioxin

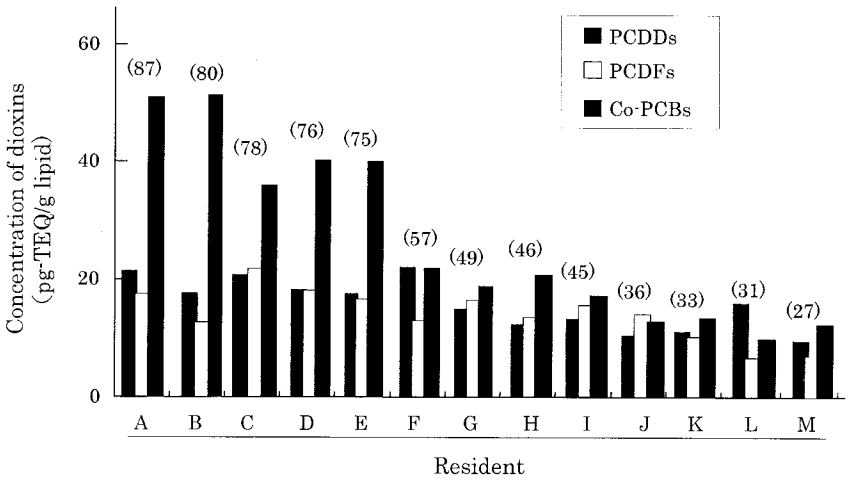

Figure 1. Serum dioxin concentration in 13 residents near an incineration facility. Values in the parentheses show total TEQ concentration

concentration and characteristics in the isomer composition and its monthly changes in subjects with higher dioxin level who were possibly intermittently exposed to dioxin. First, we researched the blood dioxin level in 13 residents near an incineration facility, which is the major source of dioxin, to find the subject with dioxin higher level. Figure 1 shows the serum dioxin concentrations in 13 residents near an incineration facility. The blood dioxin level represents TEQ of PCDD, PCDF, and Co-PCB, and the sum of TEQ of these three compounds is presented in the parenthesis as the total TEQ value. The TEQ value of PCDD and PCDF represents the sum TEQ of 7 of 2, 3, 7, 8-isomer and that of PCDF represents the sum for 10 isomers, respectively. For Co-PCB, TEQ values of 12 isomers: 4 non-ortho isomers and 8 mono-ortho isomers, were summed. Accordingly, the total TEQ value obtained by summation of TEQ of three compounds: PCDD, PCDF, and Co-PCB, represents the total value for 21 isomers. The TEQ value was presented as the concentration in 1 g of serum fat. The total TEQ value of the serum dioxin level ranged from 27 pg to 87 pg in the 13 residents and the levels were 75 pg or higher in five residents (A, B, C, D, and E). According to previous reports, the control blood dioxin level is about 50 pg or lower, and 75 pg or higher blood levels obtained in this study were relatively high. Comparing the TEQ levels of PCDD, PCDF, and Co-PCB in these five residents, the levels of PCDD and PCDF were similar in all five. In contrast, the TEQ levels of Co-PCB were higher than those of PCDD and PCDF and were 1.7-fold to 2.8-fold higher than the PCDD TEQ level. As a characteristic, comparing the TEQ levels of the three compounds, a common pattern of the presence of Co-PCB at a higher level than those of PCDD and PCDF was observed in all five residents with a dioxin level of 75 pg or higher. Although only a few studies have measured the blood levels of all 29 isomers, when the blood dioxin concentration is high in residents near an incineration facility in Japan, the ratio of some PCDF

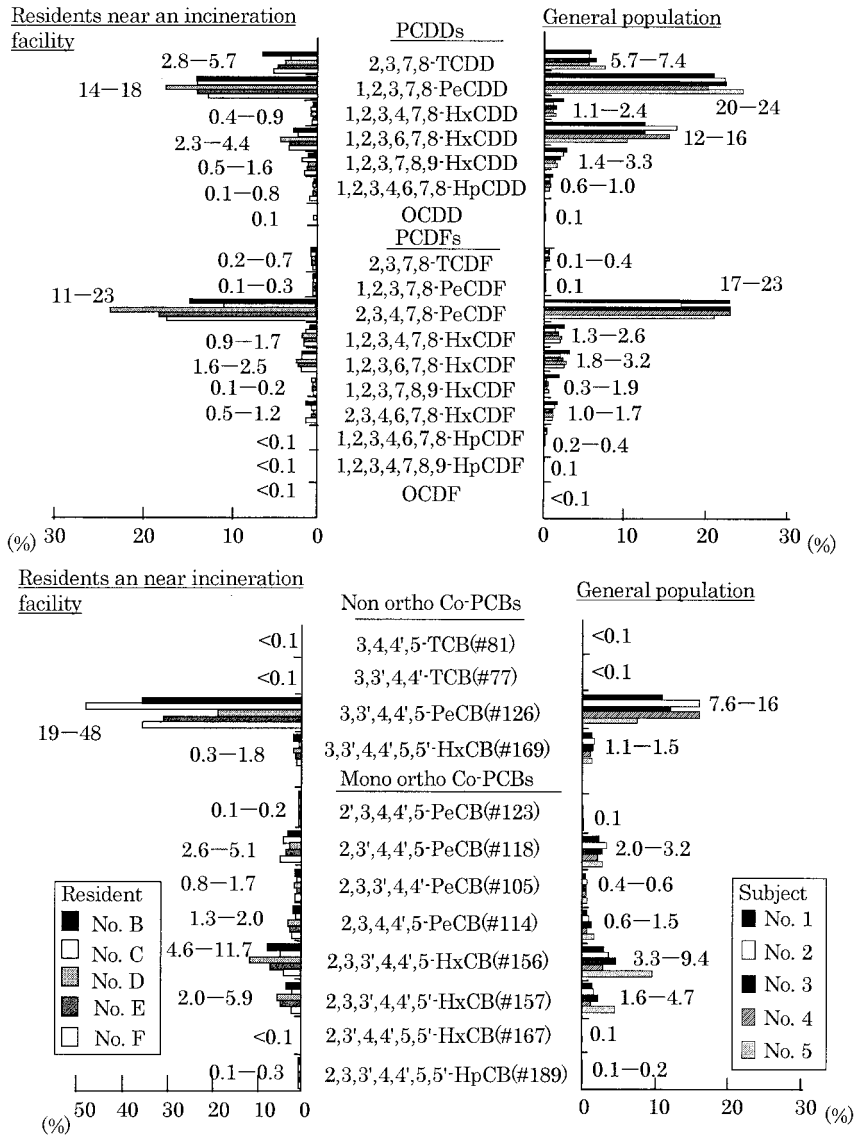

Figure 2. Comparison of the isomer composition in TEQ concentration of dioxins in serum of five residents near an incineration facility and that in the general population as control (PCDDs + PCDFs + Co-PCBs = 100 %)

isomers tends to be high among the three compounds (Kitamura 2001; Kumagai 2000). Since the results of this study were different from the results of previous studies reporting a high level of PCDF, it is necessary to investigate the relationship to the emission source, which may be the source of human contamination.

Table 2. Monthly variation in total TEQ concentration over 7 months

Resident	TEQ concentration in serum (pg · TEQ/g·lipid)								
	1st	2nd	3rd	4th	5th	6th	7th	Mean	C.V. (%)
A	84	98	100	106	79	100	69	91	15
C	59	79	113	69	46	69	49	69	33
D	66	91	100	82	53	72	87	79	20

Figure 2 shows comparison of the isomer composition in TEQ concentration of dioxins in serum of five residents near an incineration facility and that in the general population as control. The composition ratios of the isomers were calculated regarding the total TEQ value of the 29 isomers as 100%. In the five residents near an incineration facility, the major components of dioxin present in blood are four isomers: 1, 2, 3, 7, 8-PeCDD; 2, 3, 4, 7, 8-PeCDF; 3, 3', 4, 4'-PeCB; and 2, 3, 3', 4, 4', 5-HxCB; and the ratios of these isomers to the total TEQ were 14-18%, 11-23%, 19-48%, and 4.6-12%, respectively. For classification, 1, 2, 3, 7, 8-PeCDD; 2, 3, 4, 7, 8-PeCDF; 3, 3', 4, 4'-PeCB; and 2, 3, 3', 4, 4', 5-HxCB were included in PCCD, PCDF, nono-ortho PCB, and mono-ortho PCB, respectively. The four major isomers belonged to the four congeners of the classification of 29 dioxin isomers: one each in PCDD, PCDF, non-ortho PCB, and mono-ortho PCB. Of the remaining 25 isomers, 14 isomers were minor components accounting for 1.0% or less. This tendency was common in all five residents. We previously measured the blood levels five times during a three-month period in five subjects with a control dioxin level. The isomer composition ratios showed that same five isomers were major components in the five residents (Fig 2). The four of five isomers were similar when comparing the isomer composition ratios in the five residents with a high dioxin level. The four major isomers in the subjects with a high dioxin level were 1, 2, 3, 7, 8-PeCDD of PCDD; 2, 3, 4, 7, 8-PeCDF of PCDF; 3, 3', 4, 4'-PeCB of non-ortho PCB and 2, 3, 3', 4, 4', 5-HxCB of mono-ortho PCB, while the five major isomers in the subjects with a control level included these four isomers excluding 1, 2, 3, 6, 7,8-HxCDD.

The dioxin concentration was measured once a month for seven months in three of the five subjects. Table 2 shows monthly variation in TEQ concentration over 7 months. In three of the five residents described above, changes in the blood dioxin level were followed for seven months. In resident A, in whom the value in the first measurement was 84 pg, the blood dioxin level continuously increased until the fourth measurement and reached the highest level, 106 pg, during the seven-month period. Thereafter, the level decreased and increased repeatedly and reached the lowest level, 69 pg, in the seventh month. The mean level was 91 pg and the coefficient of variation (C.V.%) was 15%. The highest and lowest

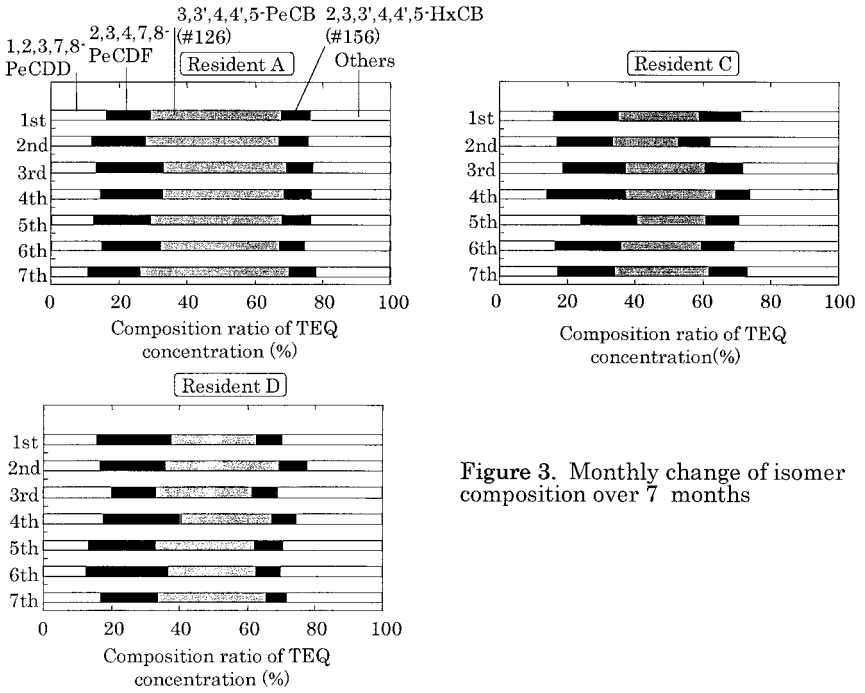

Figure 3. Monthly change of isomer composition over 7 months

blood dioxin levels during the seven-month period were 69 pg and 106 pg, respectively, and the lowest level corresponded to 65% of the highest level, showing that the dioxin concentration was maintained at a relatively high level for seven months. The highest level was 113 pg in resident C, which was higher than that in resident A, but the lowest level was 43 pg, which corresponded to 38% of the highest level. In resident D, the lowest level was less than 50% of the highest level, as in subject C, showing that the blood dioxin level varied two-fold or more in several months. In the resident with the greatest variation, the highest total TEQ value of blood dioxin reached two-fold or more of the lowest value, showing that it is necessary to measure several times to examine human exposure based on the blood level, because of the variation.

Figure 3 shows monthly changes of isomer composition over 7 months. In subject A, the four isomers described above were the major components in the first measurement. The 3, 3', 4, 4', 5-PeCB level was the highest, and the level decreased in the order of 1, 2, 3, 7, 8-PeCDD; 2, 3, 4, 7, 8-PeCDF; and 2, 3, 3', 4, 4', 5-HxCB. These four major components accounted for 80% or more of the total amount of the 29 isomers. Furthermore, the ratio was higher than 80% in seven measurements during the seven-month period, showing no marked change in the isomer composition ratios. Similar results were observed in residents C and D. The four isomers were the major components and no significant change in the composition was observed. These findings showed that although the blood level varies two-fold or more in several months, the composition ratios of 29

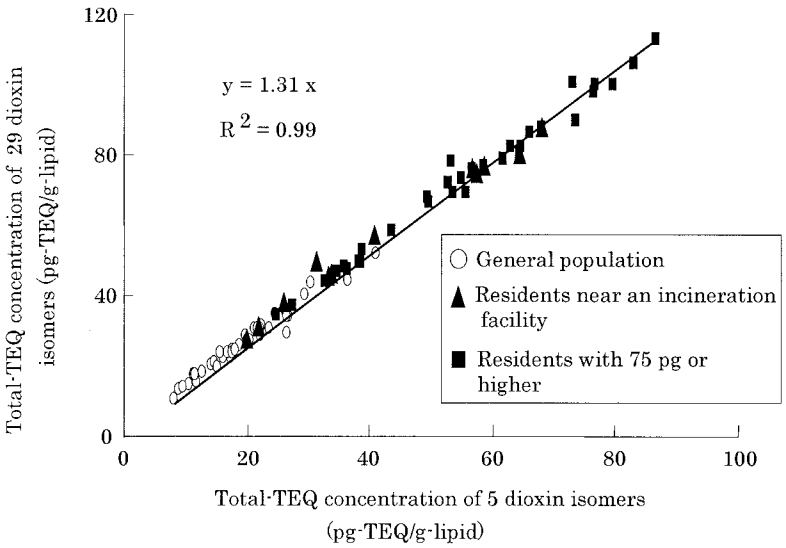

Figure 4. Relationship between total TEQ concentration of 29 dioxin isomers and that of major 5 dioxin isomers

isomers did not markedly change during the seven-month period and the four isomers described above were the major components. Therefore, constant blood isomer composition ratios were maintained during the several-month period despite the two-fold variation in the concentration in subjects with a high dioxin level, and the composition ratios were similar to those in the residents with a control dioxin level. From these results, we speculated that the total TEQ concentration of 29 isomers could be calculated from the TEQ concentrations of the five characteristic isomers present in blood. The correlation of the TEQ concentrations of the five isomers with the total TEQ concentration of 29 isomers was investigated in 59 analytical values in 13 subjects living near an incineration facility: seven measurements during the seven-month period in three subjects with a high dioxin level and five measurements during the three-month period in the five control subjects. Very high positive correlation with R^2=0.99 was obtained (Fig. 4).

Matsueda (1999) reported that the TEQ concentration of blood 2, 3, 4, 7, 8-PeCDF was correlated with the sum of TEQ of 20 isomers with R^2=0.838. Kitamura (2001) proposed that since the TEQ levels of eight isomers were correlated, they can be converted into the total TEQ concentration. Although eight mono-ortho PCB among the 29 isomers were not measured, with regard to PCDD, PCDF, and non-ortho PCB, that the total TEQ concentration of the blood dioxin corresponded to the concentrations of several isomers was reported previously, as in this study. We included eight mono-ortho Co-PCBs in this study and concluded that the TEQ value of the five isomers can be converted into the TEQ value of the 29 isomers. As described above, the blood sampling

volume can be reduced by measuring only isomers that are characteristically present at a high level in blood and converting them into the total TEQ value, which decreases the burden from the collection of blood on subjects and allows multiple tests, leading to evaluation of average exposure level.

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