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Levels of Chlordane, Oxychlordane, and Nonachlor in Human Blood

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Chlordane had been used as a termiticide for more than twenty years in Japan. It was injected into soil as an emulsion and into wood as an oil. Chlordane is stable in the environment such as in sediment. It accumulates in some species of bacteria, freshwater invertebrates, and marine fish (WHO 1984). Japanese authorities banned the use of chlordane in September 1986 because it has features described above and has possible effects on human health by continuous intake. Applying the criteria described in USEPA's guidelines for assessment of carcinogenic risk, chlordane is classified in Group B2: Probable human carcinogen (1988).

In the previous study, we investigated the levels of chlordane, oxychlordane and nonachlor in human adipose tissues (*Hirai and Tomokuni, unpublished*). Oxychlordane is one of the metabolites of chlordane in mammals (Nomeir and Hajjar 1987). Nonachlor is one of major components of technical chlordane (Sovocool et al. 1977). We detected the three compounds in all of 24 subjects. Accumulation of nonachlor was higher than that of the other two compounds. There are some reports concerning the levels of chlordane and its related compounds in the blood of pest control operators (Kawano and Tatsukawa 1982; Saito et al. 1986;Takamiya 1987;Takamiya 1990). These reports have indicated that the levels of chlordane, oxychlordane and nonachlor in blood was increased among the pest control operators. On the other hand, there is a report on the chlordane residues in normal human blood (Wariishi et al.1986) where it has been reported that the levels of oxychlordane and nonachlor in the blood of the subjects increase by the treatment of their houses with chlordane.In the present study, we investigated the levels of chlordane, oxychlordane and nonachlor in human blood. To elucidate, mainly, the

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relationships between the levels and age or sex, we randomly selected 240 subjects in consideration of age and sex. The results are described here.

MATERIALS AND METHODS

Two hundreds and sixty two blood samples of 1-5 g were obtained from 115 males and 125 females of outpatients and inpatients at Saga Medical School Hospital. Forty samples were obtained in April 1989 and two hundreds and twenty two samples were obtained in April to June 1990. Age of subjects ranged from 0 to 91 years. Mean and standard deviation of the age were 49.0 and 24.9 for male, and 48.4 and 22.7 for female.

Pretreatment of blood samples for the determination of chlordane, oxychlordane and nonachlor using GC-MS consisted of extraction with acetone and n-hexane, washing with 2% sodium sulfate solution and distilled water, drying with anhydrous sodium sulfate, concentration by rotating evaporation, clean-up with a silicagel column (Adsorbex SCX, Merck, Darmstadt, FRG) and concentration by evaporation. The capillary concentration method developed by us (Hirai and Tomokuni 1987) was applied at the final step to concentrate each sample into 20-30 μL in a capillary.

GC-MS was used to determine the total amount of *cis*-chlordane and *trans*-chlordane (chlordane, $C_{10}H_6Cl_8$), oxychlordane ($C_{10}H_4Cl_8O$), and the total amount of *cis*-nonachlor and *trans*-nonachlor (nonachlor, $C_{10}H_5Cl_9$). The apparatus used was a JEOL-DX300 double focusing GC-MS system coupled with a JEOL-DA5000 computer system (JEOL LTD, Tokyo, Japan). The MS system was operated in the mode of electron impact ionization and selected ion monitoring (SIM) of positive ions. Three fragment ions were monitored for each analyte, i.e., m/z 372.826, 374.823 and 376.820 for chlordane, m/z 386.805, 388.802 and 390.799 for oxychlordane, and m/z 406.787, 408.784 and 410.781 for nonachlor. Other analytical conditions were described elsewhere (Hirai and Tomokuni 1990). Column packing material was frequently exchanged to avoid the effects of impurities in the sample such as lipid on the elution of the analytes.

Coefficient of variation of the determination (1 ng, n=10) was less than 10%. Analytical values obtained by GC-MS were adjusted by the mean recoveries of 67%, 61% and 70% for chlordane, oxychlordane and nonachlor, obtained by adding 1 ng of each standard to 18 samples. Detection limits were 0.13 ng/g, 0.14 ng/g, and 0.06 ng/g for chlordane, oxychlordane and nonachlor.

Student's t-test preceded by F test was used to investigate the sex difference of means.

RESULTS AND DISCUSSION

Blood analysis was done twice for 14 subjects and thrice for 4 subjects at an interval of 2 days to 2 weeks. Mean of the relative deviations of the duplicate and triplicate data from their mean was 9.2% with respect to nonachlor. This fact suggests that the blood nonachlor is comparatively stable in the subject. Table 1 shows the range of chlordane and oxychlordane in blood, and the range and median of blood nonachlor for male, female and total among age classes. Median of both chlordane and oxychlordane was not tabulated because they were not detected in most subjects.

Table 1. Range of chlordane and oxychlordane, and range and median of nonachlor in ng/g in human blood of male, female and total among age classes

Age	Sex	n	chlordane range	oxychlordane range	nonachlor range	nonachlor median
0-9	male	12	n.d.	n.d.	0.07-0.46	0.16
	female	6	n.d.-0.21	n.d.	0.14-0.84	0.17
	total	18	n.d.-0.21	n.d.	0.07-0.84	0.16
10-19	male	10	n.d.	n.d.-0.24	0.14-0.96	0.19
	female	6	n.d.-0.13	n.d.-0.15	0.06-0.50	0.27
	total	16	n.d.-0.13	n.d.-0.24	0.06-0.96	0.19
20-29	male	6	n.d.	n.d.-0.26	0.18-0.63	0.22
	female	20	n.d.	n.d.-0.15	0.12-0.81	0.16
	total	26	n.d.	n.d.-0.26	0.12-0.81	0.16
30-39	male	11	n.d.	n.d.-0.34	0.18-2.30	0.27
	female	17	n.d.	n.d.-0.14	0.10-0.49	0.20
	total	28	n.d.	n.d.-0.34	0.10-2.30	0.22
40-49	male	13	n.d.	n.d.-0.20	0.28-1.37	0.45
	female	12	n.d.-0.14	n.d.-0.14	0.11-0.65	0.28
	total	25	n.d.-0.14	n.d.-0.20	0.11-1.37	0.40
50-59	male	15	n.d.-0.20	n.d.-0.17	0.14-1.29	0.39
	female	12	n.d.	n.d.-0.21	0.11-0.94	0.35
	total	27	n.d.-0.20	n.d.-0.21	0.11-1.29	0.37
60-69	male	21	n.d.-0.24	n.d.-0.59	0.11-3.40	0.44
	female	17	n.d.-0.24	n.d.-0.40	0.16-2.11	0.28
	total	38	n.d.-0.24	n.d.-0.59	0.11-3.40	0.34
70-79	male	15	n.d.	n.d.-0.53	0.19-2.73	0.44
	female	15	n.d.-0.13	n.d.-0.31	0.16-1.98	0.52
	total	30	n.d.-0.13	n.d.-0.53	0.16-2.73	0.48
80-	male	12	n.d.	n.d.-0.30	0.09-1.39	0.40
	female	13	n.d.-0.60	n.d.-0.62	0.14-3.85	0.33
	total	25	n.d.-0.60	n.d.-0.62	0.09-3.85	0.34
total	male	115	n.d.-0.24	n.d.-0.59	0.07-3.40	0.36
	female	125	n.d.-0.60	n.d.-0.62	0.06-3.85	0.28
	total	240	n.d.-0.60	n.d.-0.62	0.06-3.85	0.34

n.d.; not detected

Chlordane was detected in the subjects of 5 males and 5 females with age of 5.1 to 85.7 years. The level ranged from 0.13 to 0.60 ng/g. Trace levels of chlordane were observed in many other subjects, but they were neglected because of high blank levels. In 3 of the 10 subjects, blood chlordane levels were lower than their oxychlordane levels. In other 7 subjects, blood chlordane levels were higher than their oxychlordane levels. Nonachlor was detected in all of the 10 subjects, and its level was higher than the chlordane level. Simple correlation coefficient between the chlordane level and the nonachlor level was 0.68 indicating the statistical significance at 0.05 level. These facts suggest the existence of the difference in metabolic rate of chlordane among subjects.

Oxychlordane was detected in 11 males and 13 females with age of 14.9 to 87.4 years. The level ranged from 0.14 to 0.62 ng/g. Trace levels of oxychlordane were observed in many other subjects, but they were neglected because of low sensitivity. All of the oxychlordane level of the 24 subjects was higher than the chlordane level, but lower than the nonachlor level. Simple correlation coefficient between the oxychlordane level and the nonachlor level was 0.94 indicating the statistical significance at 0.001 level. These facts suggest that the detected oxychlordane is the metabolic product of chlordane which was accompanied by the detected nonachlor.

Nonachlor was detected in all subjects, and it ranged from 0.06 to 3.85 ng/g. Ratio of the highest level to lowest one was 64. There was an increase in median from younger class to older one (Table 1). However, its increase was as little as twofold to threefold. Median (0.34 ng/g) of all subjects was lower than that (3.6 ng/g, n=21) of the *trans*-nonachlor levels in pest control operators reported by Kawano and Tatsukawa (1982), but it was higher than the geometric means (0.09 ng/ml for male, 0.07 ng/ml for female) of *trans*-nonachlor in normal subjects reported by Wariishi et al. (1986). Blood nonachlor levels of 8 males and 5 females were higher than 1 ng/g. These subjects were 5.4% of all subjects. The rate was similar to the rate of 4.4% obtained by a survey of serum of general population of USA in 1976-80 (Murphy 1985).

Relationships between age and the nonachlor level are shown in Fig. 1 A and B, for male and female, respectively. Nonachlor was detected in the umbilical cord blood of two males of 0 year old. This finding indicates the transfer of nonachlor from mother to fetus through placenta. Nonachlor was also detected in the subjects of less than 3.5 years old who were born

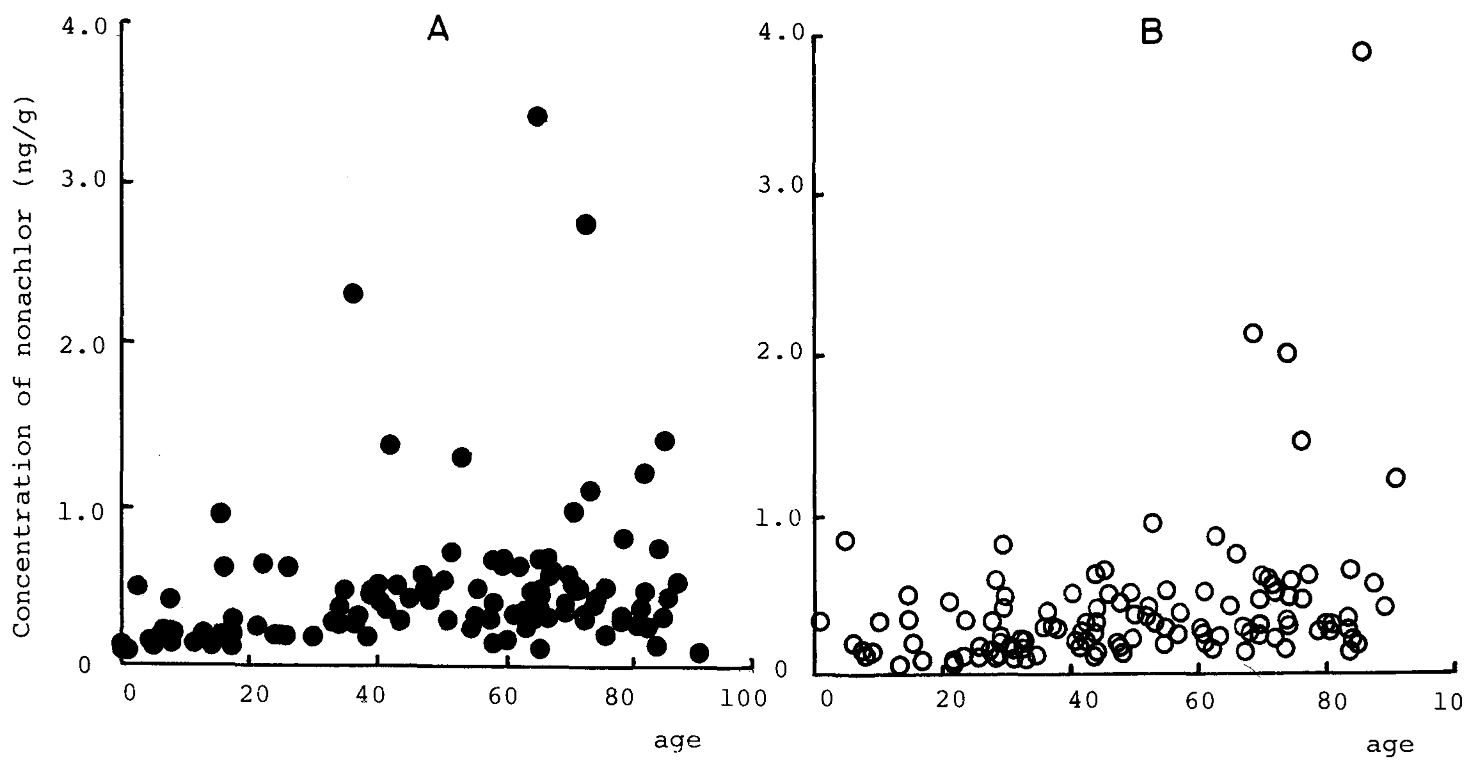

Figure 1. Relationships between the nonachlor concentration in blood and age
A, male; B, female

after the prohibition of the use of chlordane. The levels were lower in the subjects of less than 20 years old who were born after the start of the use of chlordane as a termiticide. There were many female subjects of 20 to 50 years old whose nonachlor levels were lower than the median of total subjects. The number of subjects with blood nonachlor level higher than 1 ng/g was larger in younger males than in younger females. Many subjects with blood nonachlor levels lower than the median of total subjects were observed at the age between 50 and 91. Calculated regression line and correlation coefficient were $Y=0.24+0.0046X$ and $r=0.246$, $Y=0.08+0.0064X$ and $r=0.330$, and $Y=0.16+0.0055X$ and $r=0.286$, for male (n=115), female (n=125) and total (n=240), where X, Y and r are age, nonachlor level and correlation coefficient, respectively. The correlation coefficient was significant at the level of 0.01, 0.001, and 0.001 for male, female and total, respectively.

As shown in Fig. 2, the frequency distribution of nonachlor value for total subjects was estimated to be log-normal. Geometric mean and standard deviation were -0.451 and 0.305, -0.538 and 0.301, and -0.496 and 0.306 for male, female and total, respectively. The geometric means expressed in ng/g were 0.35, 0.29 and 0.32, for male, female and total, respectively. Sex difference in the blood nonachlor levels of total subjects was smaller but significant at 0.05 level ($t=2.23$). On the other hand, the sex difference was significant at 0.001 level ($t=3.84$) when the age of the subjects was restricted between 25 and 49.

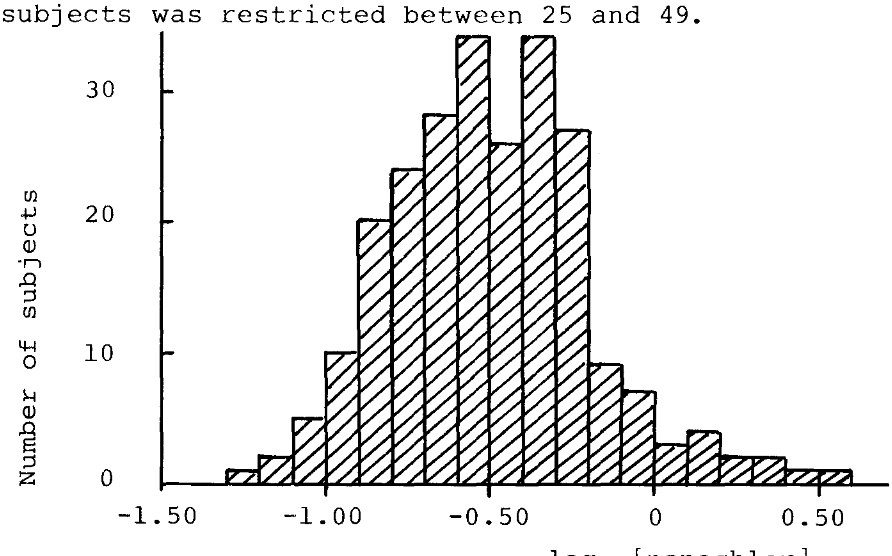

Figure 2. Frequency histogram of nonachlor concentration in human blood

Further investigation is in progress to elucidate the metabolic distribution of these compounds in human.

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REFERENCES

Hirai Y, Tomokuni K (1987) Simple sample-pretreatment for GC/MS determination of residual chlordanes in river sediments. Bunseki Kagaku, 36: 817-819.

Kawano M, Tatsukawa R (1982) Chlordanes and related compounds in blood of pest control operators (PCOs). Nippon Nogeikagaku Kaishi 56:923-929.

Murphy R, Harvey C (1985) Residues and metabolites of selected persistent halogenated hydrocarbons in blood specimens from a general population survey. Environ Health Perspect, 60:115-120.

Nomeir A A, Hajjar N P (1987) Metabolism of chlordane in mammals. Rev Environ Contam Toxicol, 100:1-22.

Saito I, Kawamura N, Uno K, Hisanaga N, Takeuchi Y, Ono Y, Iwata M, Gotoh M, Okutani H, Matsumoto T, Fukaya Y, Yoshitomi S, Ohno Y (1986) Relationship between chlordane and its metabolites in blood of pest control operators and spraying conditions. Int Arch Occup Environ Health, 58:91-97.

Sovocool GW, Lewis RG, Harless RL, Wilson NK, Zehr RD (1977) Analysis of technical chlordane by gas chromatography/mass spectrometry. Anal Chem, 49:734-740.

Takamiya K (1987) Residual levels of plasma oxychlordane and trans-nonachlor in pest control operators and some characteristic of these accumulations. Bull Environ Contam Toxicol 39: 750-755.

Takamiya K (1990) Interruption of chronic chlordane exposure and plasma residue levels in occupational workers. Bull Environ Contam Toxicol 44: 905-909.

USEPA(1988) Chlordane. Rev Environ Contam Toxicol, 104: 47-62.

Wariishi M, Suzuki Y, Nishiyama K (1986) Chlordane residues in normal human blood. Bull Environ Contam Toxicol, 36: 635-643.

WHO (1984) Environmental health criteria 34 "Chlordane" p 18, pp 49-54.

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