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Chlordane Residues in Normal Human Blood

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Determination of chlordane residues in the blood seems to be a useful means for the evaluation of contamination of humans with chlordane. However, there are only a very few reports available on this subject. Sovocool and Lewis (1975) detected oxychlordane, heptachlor epoxide and trans-nonachlor in the blood of pest control operators. Kawano and Tatsukawa (1982) detected a variety of chlordanes in the blood of termite control operators, but failed to detect chlordane residues in workers in other occupations, with the exception of one subject whose oxychlordane level exceeded the detection limit of 0.1 ppb. Barquet et al. (1981), who attempted to determine chlordane levels in the serum of normal subjects, did not present quantitative values, because the levels were below the detection limit 0.7-1.0 ppb.

In the present study, we were able to detect chlordane and its residues in fresh blood samples of normal subjects by a highly sensitive analytical technique with a detection limit of 0.01 ppb. The results are reported below with discussion of the exposure sources producing such blood levels and the process of subsequent *in vivo* metabolism of the compounds detected.

MATERIALS AND METHODS

Blood specimens were obtained from 22 male and 21 female healthy medical students, researchers and office workers of the University of Tokushima. They live in, or in the suburbs of, Tokushima City, Japan, and have had no special exposure to chlordane, with the exception of a few who were likely to have been exposed to this compound following termite control treatment of their residences within the past five years.

We used the analytical technique reported by Wariishi et al. (1984) and described briefly below.

Formic acid (5 ml) was mixed with 5 ml of each blood sample, and chlordane residues were extracted three times, each with 20 ml of n-hexane. The extract was condensed and fractionated in a Florisil column at a room temperature of about 24°C as follows: The first step of elution with 125 ml of hexane (the first 40 ml was discarded) yielded trans-nonachlor; the second step with 75 ml of 20% dichloromethane in hexane yielded cis-nonachlor, trans- and cis-chlordane and oxychlordane; and the third step with 210 ml of 30% dichloromethane in hexane (the first 80 ml was discarded) yielded heptachlor epoxide. Each fraction was condensed and injected into the 2% OV-1 column of an ECD-gas chromatograph (Shimazu Co. 8AGC). The detection limit was 0.01 ng/ml as the concentration in 5 ml of blood for all compounds. Gas chromatography-mass spectrometry (GC-MS) performed on some blood specimens resulted in similar values.

RESULTS AND DISCUSSION

The blood levels of chlordane and its residues are individually tabulated for the male subjects in Table 1 and for the females in Table 2. The tables do not include heptachlor epoxide which was detected at a high, but rather inaccurate, level in all subjects. As shown in the histogram in Figure 1, the concentrations of each compound showed a distribution close to the logarithm of the normal type. Therefore, values obtained by logarithmic conversion were used for the subsequent computation.

The blood levels of all compounds, and that of total chlordane showed no difference by sex. Table 3 shows the simple correlation coefficients among the compounds. A significant correlation was noted between trans-nonachlor and oxychlordane, between trans-chlordane and cis-chlordane, between trans-chlordane and cis-nonachlor and between cis-chlordane and cis-nonachlor.

Table 4 shows the means for each subject group divided according to each item in the questionnaire used. The group who had his/her home treated for termite control in recent years showed high oxychlordane and trans-nonachlor levels. The levels of these two compounds were also high in the group who wrote that they ate a large amount of fish.

Table 1. Blood concentration of chlordane residues in male subjects

Subject No.	Age	Termite control[a]	Concentration (ng/ml)					
			Oxy-chlor[b]	*trans*-Nona[c]	*trans*-Chlor[d]	*cis*-Chlor[e]	*cis*-Nona[f]	Total
1	56	-	0.33	0.22	0.05	0.04	0.02	0.67
2	41	+	0.72	0.20	0.01	0.01	-	0.94
3	40	-	0.12	0.10	0.04	0.02	0.03	0.31
4	52	-	0.36	0.12	0.12	0.05	0.03	0.68
5	29	-	0.11	0.08	0.04	0.03	0.02	0.28
6	38	-	0.24	0.11	0.20	0.06	0.06	0.67
7	54	+	0.39	0.29	0.11	0.07	0.05	0.92
8	38	-	0.14	0.08	0.14	0.05	0.03	0.44
9	37	?	0.09	0.05	0.02	0.02	0.01	0.18
10	42	-	0.69	0.14	0.06	0.04	0.01	0.94
11	18	-	0.14	0.04	0.11	0.06	0.03	0.38
12	20	?	0.57	0.15	0.26	0.10	0.08	1.16
13	20	?	0.12	0.11	0.14	0.09	0.04	0.50
14	32	?	0.12	0.04	0.13	0.05	0.02	0.35
15	40	-	0.13	0.12	0.13	0.08	0.04	0.49
16	22	+	0.19	0.08	0.13	0.07	0.04	0.50
17	22	-	0.20	0.04	0.20	0.06	0.05	0.55
18	21	-	0.11	0.01	0.04	0.02	-	0.18
19	22	-	0.13	0.06	0.18	0.07	0.04	0.49
20	24	?	0.22	0.12	0.16	0.09	0.05	0.65
21	22	?	0.24	0.15	0.16	0.09	0.04	0.68
22	23	?	0.11	0.13	0.17	0.07	0.04	0.52
Geometric Mean			0.20	0.09	0.09	0.05	0.03	0.51
		S.D.	1.9	2.1	2.2	1.8	1.7	1.6

a. Residence treated for termite control during the past 5 years. Yes (+), No (-), Unknown (?)

b. Oxychlordane c. *trans*-Nonachlor d. *trans*-Chlordane

e. *cis*-Chlordane f. *cis*-Nonachlor

Table 2. Blood concentration of chlordane residues in female subjects

Subject No.	Age	Termite control[a]	Concentration (ng/ml)					
			Oxy-chlor[b]	trans-Nona[c]	trans-Chlor[d]	cis-Chlor[e]	cis-Nona[f]	Total
1	22	-	0.15	0.04	0.05	0.03	0.01	0.27
2	24	+	0.27	0.15	0.11	0.09	0.04	0.65
3	56	?	0.14	0.11	0.10	0.06	0.03	0.44
4	21	?	0.34	0.11	0.15	0.07	0.04	0.71
5	20	?	0.28	0.09	0.15	0.07	0.03	0.61
6	20	-	0.25	0.16	0.12	0.06	0.03	0.62
7	22	?	0.12	0.01	0.05	0.03	0.02	0.22
8	24	-	0.10	0.09	0.04	0.03	0.01	0.28
9	22	-	0.36	0.08	-	-	-	0.12
10	22	-	0.49	0.04	0.07	0.05	0.01	0.66
11	22	?	0.19	0.07	0.11	0.07	0.03	0.46
12	20	+	0.75	0.09	0.15	0.08	0.04	1.12
13	21	+	0.23	0.05	0.07	0.04	0.02	0.41
14	21	-	0.41	0.08	0.08	0.06	0.01	0.63
15	20	-	0.23	0.04	0.08	0.04	0.02	0.41
16	21	-	0.08	0.06	0.12	0.07	0.03	0.36
17	21	-	0.56	0.08	0.17	0.09	0.04	0.94
18	21	-	0.25	0.09	0.17	0.07	0.05	0.63
19	21	-	0.12	0.06	0.14	0.09	0.05	0.45
20	21	+	0.31	0.12	0.20	0.12	0.07	0.82
21	21	-	0.18	0.07	0.22	0.14	0.07	0.49
Geometric mean			0.23	0.07	0.11	0.06	0.03	0.46
		S.D.	1.8	1.9	1.6	1.6	2.0	1.7

a. Residence treated for termite control during the past 5 years. Yes (+), No (-), Unknown (?)

b. Oxychlordane c. *trans*-Nonachlor d. *trans*-Chlordane

e. *cis*-Chlordane f. *cis*-Nonachlor

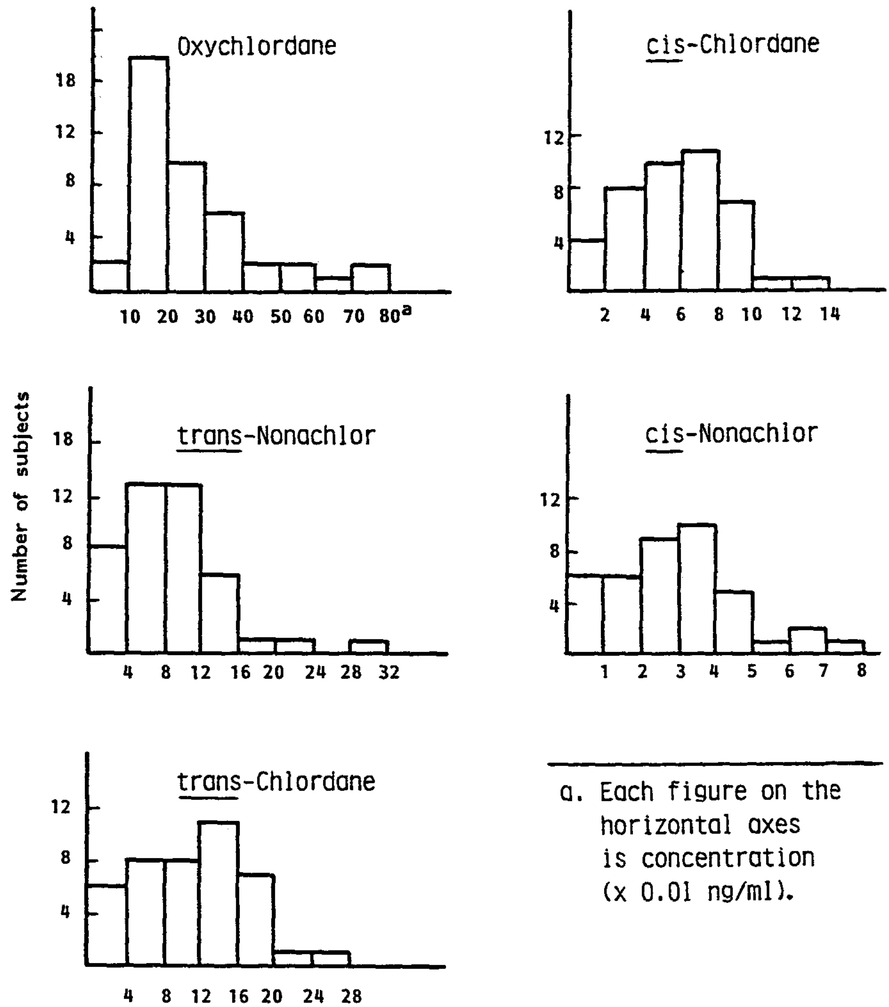

Figure 1. Distribution of the concentrations of chlordane residues in the blood.

Food seems to be the main source of chlordane residues which were detected at low levels in the blood of all of the healthy normal subjects with no chance of chlordane exposure.

The rate of detection of chlordane residues in food items is generally low, and the concentration, if any, is also low. For example, US EPA (1976) shows that the market basket survey conducted in the U.S.A. during the years 1963-1969 revealed detection rates of 1% or below, with mean values of 1-5 ppb. With respect to fish, the level of detection was from zero to

Table 3. Coefficients of correlation among chlordane residues in the blood

	trans-Nonachlor	trans-Chlordane	cis-Chlordane	cis-Nonachlor
Oxy-chlordane	0.455[a]	0.112	0.196	0.001
trans-Nonachlor	-	0.002	0.003	0.003
trans-Chlordane	-	-	0.916[a]	0.832[a]
cis-Chlordane	-	-	-	0.774[a]

a. Significant at p = < 0.01

Table 4. Mean concentration in each group divided according to the questionnaire items

		Concentration (ng/ml)					
Group	N	Oxy-chlordane	trans-Nonachlor	trans-Chlordane	cis-Chlordane	cis-Nonachlor	Total
Residence treated for termite control during the past 5 years							
Yes	7	0.32[a]	0.12[b]	0.09	0.05	0.03	0.66
No	24	0.20	0.07	0.10	0.05	0.03	0.45
Possible contamination of the area of the residence due to agricultural chemicals							
Yes	22	0.22	0.09	0.09	0.05	0.03	0.48
No	17	0.21	0.07	0.11	0.06	0.03	0.48
Frequency of fish intake							
High	11	0.23	0.11[b]	0.10	0.05	0.03	0.52
Low	12	0.20	0.06	0.10	0.06	0.03	0.45
Frequency of meat intake							
High	17	0.18	0.06	0.10	0.05	0.03	0.42
Low	2	0.17	0.07	0.05	0.03	0.02	0.34

a. Significantly different from the "No" group ($p < 0.01$).

b. Significantly different from the "Low" group ($p < 0.05$).

0.046 mg/kg, as reported by Frank et al. (1978), and Butler and Schutzmann (1978) reported a detection rate of 3%. On the other hand, Yamagishi et al. (1981) detected chlordane residues in all fish caught in Tokyo Bay and its feeders and other bays in Japan. The level of total chlordane was 11-225 ppb in *Acanthogobius flavimanus* and 9-489 ppb in *Zacco platypus*. The relatively high level of this agent in the bodies of inshore fish may be due to the large amount of chlordane used in a given area as a result of crowding of the pertinent districts with houses. Since food items, except for fish, have not been sufficiently analyzed with regard to chlordane residues in Japan, it can only be speculated that fish is the major source of the contamination of humans with chlordane in Japan, because, as an additional factor, the Japanese eat fish be preference. This is supported by the fact that, in the present study, the mean blood levels of the residues were high among the subjects with a high frequency of fish intake.

Technical chlordane consists of more than 45 components including, as major ones, *trans*- and *cis*-chlordane and *trans*-nonachlor at about 24%, 9% and 7%, respectively (Sovocool et al. 1977). These major components are relatively stable in soil, water, air and plants (US EPA, 1976). In mammals, *trans*-nonachlor is sparingly metabolized, whereas *trans*- and *cis*-chlordane are freely metabolized into oxychlordane (Street and Blau, 1972; Barnett and Dorough, 1974; Tashiro and Matsumura, 1977). These compounds seem to be sparingly metabolized in fish (Feroz and Kahn, 1979). As a result, oxychlordane and *trans*-nonachlor are major residues in livestock products such as meat and cow's milk, and fish contain *trans*- and *cis*-chlordane as major residues. Yamagishi et al. (1981) compared the component patterns of residues in cat and dog adipose tissue with that in fish and found clear differences.

Although the type and amounts of chlordanes incorporated into the human body depend on the quality and amount of food items eaten, after absorption they are thought to be subjected to a metabolism similar to that in the above-mentioned mammals. Oxychlordane and/or *trans*-nonachlor have been detected and determined quantitatively in human adipose tissue (Kutz et al. 1976, 1977) and human milk (Strassman and Kutz, 1977; Mes and Davies, 1978; Takahashi et al. 1981; Savage et al. 1981). In our present study too, oxychlordane and *trans*-nonachlor showed a higher level than did the other residues in the blood of normal individuals. These two levels showed an elevation in the group of subjects whose residences had undergone termite control in recent years and the group with a large intake of fish. Moreover, a significant correlation was noted between the two compounds. The effect of termite control is thought to originate in contamination with household dust (Starr et al. 1974) and/or the ambient air (Wright and Leidy, 1982).

Oxychlordane and *trans*-nonachlor thus appear to be major terminal residues in the blood. On the other hand, the level of *trans*-chlordane was similarly high, but this pattern in the blood was somewhat different from that in adipose tissue or breast milk, an issue which remains to be elucidated. It is of interest to note that the *trans*-chlordane level showed a high correlation with the *cis*-chlordane and *cis*-nonachlor levels.

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