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Levels of Chlordane, Oxychlordane, and Nonachlor on Human Skin and in Human Blood

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Chlordane had been used as a termiticide for more than twenty years in Japan. The characteristic features of chlordane are that it is stable in an environment such as sediment (Oloffs et al. 1978, Hirai and Tomokuni 1989) and that its bioaccumulation in some species of bacteria, invertebrates, and fish is large (WHO 1984).

Although the use of chlordane was prohibited in Japan in September 1986, chlordane and its related compounds are still detected in human body such as in blood and in adipose tissues (Hirai and Tomokuni 1991a, 1991b, Sasaki et al. 1991a). These facts indicate the slow elimination of the compounds from human body (WHO 1984, Nomeir and Hajjar 1987, USEPA 1988, Takamiya 1990) and/or successive intake. Wariishi and Nishiyama (1989) observed the progression of chlordane contamination in humans by blood and sebum analysis. They concluded that skin lipid might be a unique and available sample source for evaluating direct exposure to technical chlordane. Sasaki et al. (1991b) reported that *trans*-nonachlor and *trans*-chlordane in skin lipids was satisfactory for detecting dermal exposure to chlordane and that oxychlordane in skin lipids reflected its accumulation in the body. In the present study, we investigated the levels of chlordane, oxychlordane, and nonachlor on human skin and in human blood. The data obtained are discussed mainly in consideration of age and sex.

MATERIALS AND METHODS

The subjects were outpatients at Saga Medical School Hospital in January to December 1991 (Table 1). The purpose of the present study and the procedures were explained to about 500 of subjects, and informed consent was obtained from 248 of males and from 227 of females to wipe skin surface of antebrachium and/or to use for analyzing rest of the blood utilized for hematological examination. The average weight and height of the subjects were 60.1±12.6 kg (mean±standard deviation) (n=244) and 165±11 cm (n=241) for males, and 50.0±8.3 kg (n=225) and 154±8 cm (n=224) for females. The

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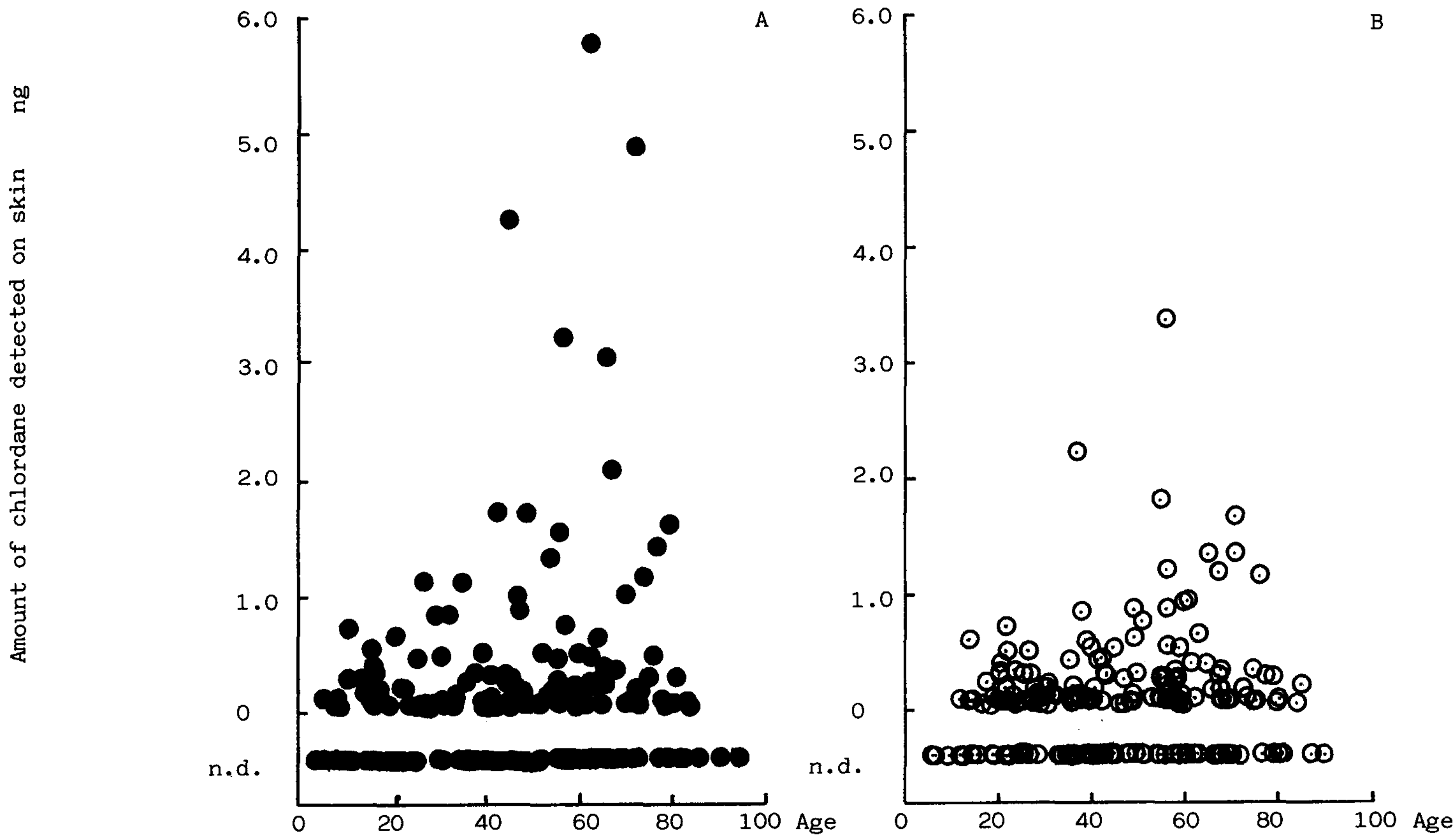

Figure 1. Relationships between age and the amount of chlordane detected on human skin
A;male (n=228), B;female (n=204)

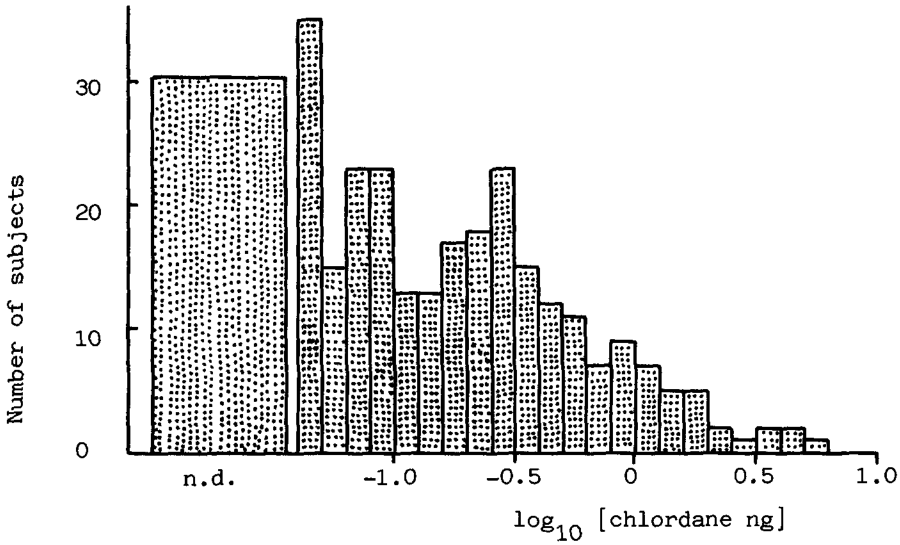

Figure 2. Histogram of the amount of chlordane detected on skin

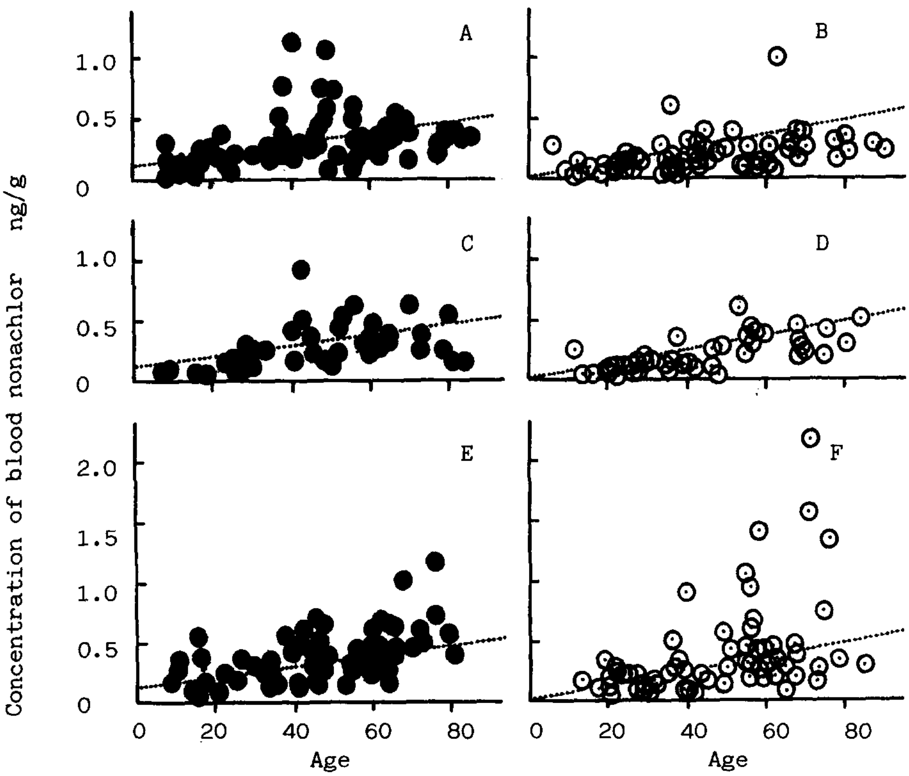

Figure 3. Relationships between age and the concentration of blood nonachlor screened by the amount of skin chlordane detected A,C,E;male, B,D,F;female
Skin chlordane:A,B;n.d., C,D;0.04≤ <0.10ng, E,F;≧0.10ng

subjects were different from those in our previous study (Hirai and Tomokuni 1991a) except two males with age of 8.8 and 65.6 and one female with age of 14.4.

Table 1. The number and age of subjects

Sample	sex	n	range	mean age	SD
Skin	male	228	3.7-94.4	46.8	21.0
	female	204	5.8-89.8	44.8	19.7
	total	432	3.7-94.4	45.9	20.4
Blood	male	192	7.4-86.0	46.1	19.9
	female	191	5.8-89.8	45.1	19.3
	total	383	5.8-89.8	45.6	19.6
Skin and Blood	male	177	7.4-86.0	46.1	20.1
	female	171	5.8-89.8	44.7	19.6
	total	348	5.8-89.8	45.4	19.9

The sampling point and method for skin analysis were selected so as to reduce physical and mental burden on subjects. Fat-free cotton for skin sampling was washed with 8 ml of acetone-hexane (3:7) and dried before use. Skin surface sample was collected by wiping 25 cm^2 of antebrachium with 2 cm x 2 cm of the fat-free cotton soaked with 0.5 ml of 70% ethyl alcohol water solution. The sampling was done once with three strokes of wiping.The cotton was packed into a column (8mm i.d.). The column was eluted with 10 ml of acetone-hexane (1:9). The eluate was washed with 2 ml of 2% sodium sulfate solution and distilled water, and dried with anhydrous sodium sulfate. The dried solution was concentrated by rotating evaporator into 0.5 ml hexane solution. After clean-up with a silicagel column (Adsorbex SCX(400mg), Merck, FRG), the eluate was concentrated into 20-30 µl in a capillary (Hirai and Tomokuni 1987). The method for blood analysis was described elsewhere (Hirai and Tomokuni 1991a).

GC-MS was used to determine total amount of *cis*-chlordane and *trans*-chlordane, oxychlordane, and total amount of *cis*-nonachlor and *trans*-nonachlor as described in the report on the levels of these compounds in human blood (Hirai and Tomokuni 1991a). Coefficient of variation of the determination (1ng, n=10) was less than 10%. The recovery of *cis*-chlordane, oxychlordane, and *trans*-nonachlor obtained by adding 1 ng of each standard to cotton was about 50% for the three compounds. Detected amounts are described in the results. Detection limits were 0.04 ng, 0.08 ng, and 0.02 ng for chlordane, oxychlordane, and nonachlor, respectively.

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

RESULTS AND DISCUSSION

Chlordane on the skin surface was detected in 132 males with age of 7.4 to 80.4 and in 127 females with age of 11.6 to 84.7. The amounts ranged from 0.04 to 5.76 ng for males and from 0.04 to 3.37 ng for females. Nonachlor was detected in 127 males and in

118 females. The amounts ranged from 0.02 to 1.91 ng for males and from 0.02 to 1.19 ng for females. We could not detect oxychlordane. Calculated regression lines and correlation coefficients between chlordane and nonachlor were Y=0.02+0.34X r=0.99 for males (n=123) and Y=0.01+0.35X r=0.98 for females (n=117), where X, Y, and r are amount of chlordane detected, that of nonachlor, and correlation coefficient. Simple correlation coefficients were statistically significant at 0.001 level. Such a high correlation was observed by Sasaki et al. (1991b). No significant difference was observed between males and females.

Relationships between age and the amount of chlordane detected on skin are shown in Fig. 1. Calculated regression lines and correlation coefficients were Y=0.14+0.0070X r=0.17, Y=0.14+ 0.0041X r=0.17, and Y=0.13+0.0058X r=0.16, for males (n=132), females (n=127), and total (n=259), where X, Y, and r are age, amount of chlordane detected, and correlation coefficient. The slope for males was larger than that for females. The correlation coefficient was smaller, but significant for total at 0.05 level.

Histogram of skin chlordane is shown in Fig. 2. An obscure peak was observed at higher level than 0.10 ng with a maximum at 0.25 to 0.32 ng. Such a peak was also observed in the histogram of skin nonachlor, where the peak located at 0.10 to 0.12 ng. Medians of the amounts of chlordane detected were 0.06, 0.07, and 0.06 ng for males, females, and total. Medians of skin nonachlor were 0.02 ng for males, females, and total. No remarkable difference was observed in the median levels of skin chlordane and skin nonachlor between males and females.

Table 2. Regression equations for the linear relationship among chlordane, oxychlordane, and nonachlor in human blood (ng/g)

X	Y	sex	Regression equation	r	n
chlordane	oxychlordane	male	Y=0.11+0.81X	0.36	26
		female	Y=0.07+1.72X	0.69*	25
chlordane	nonachlor	male	Y=0.57+0.49X	0.08	26
		female	Y=0.27+6.16X	0.71*	25
oxychlordane	nonachlor	male	Y=0.44+0.98X	0.37	26
		female	Y=0.10+3.19X	0.92*	25

* p<0.001

Blood chlordane was detected in 46 males with age of 10.8 to 80.8 and in 47 females with age of 19.8 to 86.8. The concentration ranged from 0.04 to 0.19 ng/g for males and from 0.04 to 0.24 ng/g for females. Oxychlordane was detected in 54 males with age of 9.3 to 86.0 and in 41 females with age of 11.6 to 86.8. The concentration ranged from 0.08 to 0.47 ng/g for males and from 0.08 to 0.70 ng/g for females. Nonachlor was detected except only one female with age of 28.1. The concentration ranged from 0.02 to 1.16 ng/g for males and from 0.02 to 2.17 ng/g for females. The concentration of 4 males and 8 females were higher than 1.00 ng/g. These subjects were 3.1% of total (n=383). All of chlordane, oxychlordane, and nonachlor was detected in 26 males with age of 15.6 to 79.3 and in 25 females with age of 35.7 to 86.8. Linear regression equations among these compounds are shown in Table 2. Intercepts were smaller in females than in males. Slopes were larger in females than in males. Correlation coefficients were smaller in males than in females. We suppose from these results the existence of the difference in metabolic rate of these compounds between males and females.

Calculated regression lines and correlation coefficients between age and blood nonachlor were $Y=0.12+0.0042X$ $r=0.41$, $Y=-0.007+0.0063X$ $r=0.43$, and $Y=0.06+0.0052X$ $r=0.41$, for males (n=192), females (n=190), and total (n=382). The correlation coefficients were significant at 0.001 level. Geometric mean concentrations were 0.26, 0.20, and 0.23 ng/g for males, females, and total, respectively. The sex difference was significantly detected at 0.001 level (t=3.35).

Table 3. The levels of blood nonachlor (ng/g) screened by the amount of skin chlordane detected (ng)

Skin chlordane		sex	n	age mean(SD)	Blood nonachlor g. mean (-SE,+SE)	
n.d.		male	72	45.8(20.6)	0.24(0.22,0.26)	**
		female	63	43.9(20.5)	0.15(0.14,0.17)	
0.04≦	<0.10	male	42	45.3(20.6)	0.22(0.19,0.24)	*
		female	44	42.8(19.8)	0.16(0.14,0.18)	
≧0.10		male	63	46.8(19.1)	0.33(0.31,0.36)	
		female	64	46.8(18.2)	0.27(0.24,0.30)	

** $p<0.001$ t=3.56, * $p<0.10$ t=1.85

Figure 3 shows the relationships between age and nonachlor concentration in human blood screened by the detected amount of skin chlordane. Dotted lines show the regression lines for males (n=177) (Fig. 3 A,C,E) and females (n=170) (Fig. 3 B,D,F). There were some subjects with age of 30 to 50 at the groups of lower level of skin chlordane (Fig. 3 A,B) whose blood nonachlor were markedly higher than the level of the regression lines. The number was larger in male than in female. No apparent increase in concentrations of blood nonachlor was accompanied by the increase in the level of skin chlordane (Fig. 3 C,D). Somewhat increase in concentrations of blood nonachlor was observed at the groups of higher level of skin chlordane (Fig. 3 E,F) especially in females of age between 50 to 80. There were some boys and girls whose nonachlor concentrations in blood were as high as the level of middle age.

The geometric mean levels of blood nonachlor screened by the detected amount of skin chlordane are shown in Table 3. The level was higher in males than in females in every group. The sex difference was larger in the groups of the subjects with lower level of skin chlordane, especially in the n.d. groups where the significance was at 0.001 level. The increase in the levels of blood nonachlor at the groups of higher level of skin chlordane may be explained by the direct exposure to nonachlor in the subjects of higher levels of skin chlordane as reported by Wariishi and Nishiyama (1989), and Sasaki et al. (1991b).

Table 4. Regression equations for the linear relationship between the amount of chlordane or nonachlor detected on human skin (ng), and concentration of chlordane, oxychlordane, and nonachlor in human blood (ng/g)

X	Y	sex	Regression equation	r	n
Skin chlordane	Blood chlordane	male	Y=0.07+0.0005X	0.02	33
		female	Y=0.05+0.043X	0.47**	31
Skin chlordane	Blood oxychlordane	male	Y=0.13+0.022X	0.38*	32
		female	Y=0.13+0.061X	0.37*	39
Skin nonachlor	Blood nonachlor	male	Y=0.31+0.19X	0.29**	97
		female	Y=0.17+1.10X	0.58***	100

*** $p<0.001$, ** $p<0.01$, * $p<0.05$

Table 4 shows the linear relationships between the amount of chlordane or nonachlor detected on human skin and the concentration of chlordane, oxychlordane and nonachlor in human blood. Intercepts were higher in males than in females with respect to the correlation between skin nonachlor and blood nonachlor. Slopes were larger in females than in males. Correlation coefficients were larger in females than in males except the relationship between skin chlordane and blood oxychlordane.

The levels of skin chlordane and nonachlor, and blood nonachlor as determined monthly are shown in Table 5. The level of skin chlordane was minimum in March and maximum in July. The level of blood nonachlor was maximum in January and minimum in July. Fluctuation (maximum/minimum) of the monthly level was about four for skin chlordane and nonachlor, and it was about two for blood nonachlor. Mean temperature is minimum in January to February and maximum in August. We have a rainy season in June to July. Mean relative humidity is minimum in February to March and maximum in June to July. Most people wear clothes with half-length sleeves in July to September. The level on skin and in blood may be affected partially by the temperature and the relative humidity, and by the clothes as has been pointed out by Wariishi and Nishiyama (1989). No apparent correlation was observed between the monthly levels of skin chlordane and blood nonachlor, or skin nonachlor and blood nonachlor.

The peak observed in the histogram of skin chlordane as well as the component of skin chlordane and nonachlor suggest the existence of direct exposure to chlordane residues. About 38% (163/432) of subjects was included in the peak with the level of 0.1 ng or higher (Fig. 2). From the rate we assume they are exposed to chlordane residues in the house where they live in. The main origin of chlordane, oxychlordane, and nonachlor detected in the blood of the other subjects may be food (Matsumoto et al. 1987, Taguchi et al. 1989) or water. There was more than 50 times

Table 5. The levels of chlordane and nonachlor on human skin (ng), and nonachlor in human blood (ng/g) as determined monthly

Month Sampling period	sex	n	age mean(SD)	Skin chlordane median	Skin nonachlor median	Blood nonachlor g.mean(-SE,+SE)
January 7-11	male	17	53.2(17.3)	0.23	0.09	0.34(0.29,0.39)
	female	12	44.0(21.3)	0.07	0.03	0.25(0.21,0.31)
	total	29	49.4(19.6)	0.09	0.05	0.30(0.26,0.34)
February 4-8	male	17	47.7(17.5)	n.d.	n.d.	0.26(0.22,0.30)
	female	18	37.7(18.1)	0.06	0.02	0.21(0.18,0.24)
	total	35	42.5(18.5)	n.d.	n.d.	0.23(0.21,0.26)
March 4-7	male	10	46.1(24.8)	n.d.	n.d.	0.27(0.19,0.36)
	female	14	49.0(18.9)	n.d.	n.d.	0.19(0.15,0.24)
	total	24	47.8(21.6)	n.d.	n.d.	0.22(0.18,0.26)
April 9-12	male	15	43.2(20.6)	n.d.	n.d.	0.26(0.22,0.30)
	female	13	46.7(18.6)	0.08	0.03	0.23(0.18,0.29)
	total	28	44.8(19.8)	0.05	n.d.	0.24(0.21,0.28)
May 13-16	male	19	43.0(18.0)	0.05	0.02	0.21(0.17,0.26)
	female	17	40.0(21.1)	0.07	0.03	0.20(0.15,0.26)
	total	36	41.6(19.6)	0.05	0.02	0.21(0.17,0.24)
June 10-14	male	13	49.0(18.5)	0.08	0.03	0.36(0.31,0.40)
	female	22	43.3(15.7)	0.05	0.02	0.17(0.15,0.20)
	total	35	45.4(17.0)	0.05	0.02	0.23(0.20,0.25)
July 8-11	male	17	44.6(21.3)	0.10	0.02	0.21(0.18,0.25)
	female	15	47.4(19.4)	0.07	0.03	0.11(0.08,0.15)
	total	32	45.9(20.5)	0.09	0.03	0.16(0.13,0.19)
August 5-8	male	14	42.7(20.3)	0.07	0.02	0.23(0.20,0.26)
	female	20	40.9(19.3)	0.08	0.03	0.20(0.18,0.23)
	total	34	41.6(19.8)	0.07	0.03	0.21(0.19,0.23)
September 9-12	male	14	48.0(22.0)	0.08	0.03	0.28(0.23,0.34)
	female	14	50.0(23.1)	0.09	0.04	0.20(0.16,0.25)
	total	28	49.0(22.6)	0.08	0.03	0.24(0.21,0.27)
October 14-17	male	22	47.5(19.2)	0.09	0.03	0.28(0.24,0.33)
	female	11	53.1(14.7)	0.08	0.03	0.25(0.20,0.31)
	total	33	49.4(18.0)	0.09	0.03	0.27(0.24,0.31)
November 11-12 14-15	male	9	47.1(22.5)	n.d.	n.d.	0.22(0.18,0.28)
	female	8	47.8(12.9)	0.09	0.04	0.16(0.12,0.23)
	total	17	47.4(18.6)	0.05	0.02	0.19(0.16,0.24)
December 9-13	male	10	37.9(16.8)	0.09	0.03	0.28(0.23,0.33)
	female	7	45.8(24.1)	n.d.	n.d.	0.15(0.13,0.18)
	total	17	41.2(20.5)	0.06	0.02	0.22(0.19,0.25)

difference in the levels of blood nonachlor in the subjects with non detectable levels of skin chlordane.

Further investigations are in progress to elucidate the levels of chlordane, oxychlordane, and nonachlor in human.

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