Bull. Environ. Contam. Toxicol. (1995) 54:855–862

Environmental Contamination and Toxicology

Chlordane Compounds and Metabolite Residues in Termite Control Workers' Blood

F. Jitunari, F. Asakawa, N. Takeda, S. Suna, Y. Manabe

Department of Hygiene and Public Health, Kagawa Medical School 1750-1, Ikenobe, Miki-cho, Kagawa 761-07, Japan

Received: 20 June 1994/Accepted: 4 December 1994

Termites harm a wide range of materials, but they have a dominant predilection for wood. In Japan, where there are many wooden buildings, damages caused by termites are a major problem. Chlordane was used primarily as termiticides for extermination of termites and prevention of termite-caused damages. However, environmental contamination by Chlordane was later demonstrated (Miyazaki et al., 1980a; Miyazaki et al., 1980b; Yamagishi et al., 1981) as by other organic chlorine agricultural chemicals, i.e., DDT, BHC etc., and the use of Chlordane was prohibited in September, 1986.

Individuals engaged in termite control operations using Chlordane were reported to be exposed to considerable levels of Chlordane (Kawano and Tatsukawa, 1982; Saito et al., 1986; Takamiya, 1987). Since Chlordane is poorly degradable and highly accumulable, these workers are considered to need follow-ups including biological monitoring even after the discontinuation of the use of the chemical.

Gas chromatography (GC) is effective for the analysis of chlordane compounds and metabolites (chlordanes), but simultaneous analysis of these chlordanes was impossible by the conventional method using a packed column. Procedures such as fractionation by column chromatography or the use of two or more packed columns for different compounds were needed (Takeda et al., 1984), which made the analytical procedure complex.

We developed a method for simultaneous analysis of chlordanes in blood by GC using a wide bore capillary column (WBC column), which has both the high separation ability of the capillary column and the ease of handling of the packed column, to be used for biological monitoring. We measured the blood levels of chlordanes in termite control workers who used Chlordane by this

Correspondence to: F. Asakawa

method and observed that the blood chlordane levels generally decreased after discontinuation of the use of Chlordane but that trans-nonachlor, oxychlordane, and heptachlorepoxide were detected at high levels even several years after they stopped using the chemical.

MATERIALS AND METHODS

Three types of WBC columns, namely a high-polarity column (DB-WAX), a middle-polarity column (DB-17), and a low-polarity column (DB-5) of J & W Co., were evaluated. Extraction of chlordanes from blood was made on the basis of the method by Takeda et al. (1984) using an extrelute column (Extrelute 20, Merck), and clean-up of the extracted samples was attempted by the use of sep-pak florisil (Waters) (Sapp, 1989) instead of florisil column chromatography.

A total of 7 compounds, namely cis- and trans-chlordane, which are the primary components of the termiticide, cis- and trans-nonachlor and heptachlor, which are contained as contaminants in the termiticide, oxychlordane, which is primary metabolite of chlordane, and heptachlorepoxide, which is primary metabolite of heptachlor (Tashiro and Matsumura, 1977; Tashiro and Matsumura, 1978), were measured. The analytical procedure that we have developed is described below.

Five grams of whole blood and 15 ml of distilled water were mixed well by shaking, and the mixture was infused into the extrelute column. The column was allowed to stand for 20 minutes, and chlordanes were eluted with 100 ml of n-hexane containing 5% diethyl ether. The eluate was condensed at less than 45°C with decompression to about 5 ml. The concentrated sample was infused into sep-pak florisil, which was washed in advance with 10 ml of n-hexane. Chlordanes were eluted with 10 ml of n-hexane containing 1% diethyl ether, and the eluate was condensed similarly to less than 1 ml. The sample volume was adjusted with n-hexane, and 2μl was infused into GC mounted with a WBC column (DB-WAX) for measurement. The detection limit by this procedure for each compound or metabolite was 0.2 ppb in blood. The GC apparatus was a Shimadzu GC-7A (with ECD), to which a Shimadzu chromatopack C-R6A (data recorder) was connected. The reagents used were commercial preparations for analysis of residual agricultural chemicals. As standards, heptachlor, cis- and trans-chlordane, and heptachlorepoxide of Wako Pure Chemical, and oxychlordane and cis- and trans-nonachlor of Nanogen Co., were used. All standard solutions were prepared by dissolving the compounds with n-hexane.

The subjects were 8 workers of a termite control enterprise. Blood samples

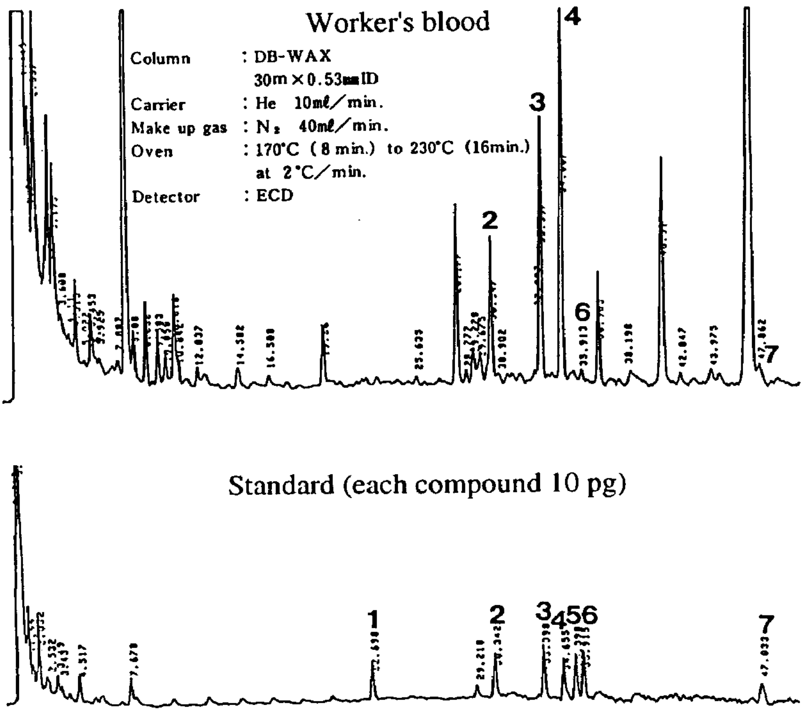

Figure 1 Gas chromatograms' examples of chlordanes on wide bore capillary column

1: heptachlor, 2: oxychlordane, 3: *trans*-nonachlor, 4: heptachlorepoxide, 5: *trans*-chlordane, 6: *cis*-chlordane, 7: *cis*-nonachlor

obtained at annual physical examinations (1987-1990) were analyzed. The period in which the subjects were engaged in termite control before the ban of the use of Chlordane (1986) ranged from 1 to 20 years.

RESULTS AND DISCUSSION

The separation of chlordanes by three WBC columns different in polarity was studied. The peaks of oxychlordane and heptachlorepoxide overlapped each other completely with the low-polarity column, and the separation of *trans*-chlordane and *trans*-nonachlor was not sufficient with the middle-polarity column. On the other hand, with the high-polarity DB-WAX column, the 7 compounds were separated nearly completely (Fig.1), suggesting that their simultaneous analysis is possible. This eliminated the necessity of the use of different columns for different chlordanes or their fractionation by column chromatography in the conventional method using packed column GC, and the analytical procedure could be simplified. Concerning the pretreatment, According to the reports of Takeda et al. (1984)

and Sapp (1989), three extraction media, namely n-hexane, n-hexane containing 1% diethyl ether, and n-hexane containing 5% diethyl ether, were tested prior to the use of the extrelute column for extraction of chlordanes from blood and the use of sep-pak florisil for clean-up of the extracted sampled. As a result, chlordanes could be recovered by nearly 100% from 5 g of whole blood by the use of 100 ml of n-hexane containing 5% diethyl ether for elution from the extrelute column and 10 ml of n-hexane containing 1% diethyl ether for elution from sep-pak florisil. Therefore, chlordanes were measured in the blood of termite control workers under these conditions. As observed in the gas chromatogram shown in Fig. 1, the seven compounds in blood could be analyzed in one run using a single column.

Table 1 shows the results of analysis blood chlordanes in 1987, 1 year after discontinuation of the use of Chlordane. Chlordanes were detected in most workers, and the blood total-chlordane concentration (total of the 7 compounds) was N.D.-21.81 ppb. Of the components of the termiticide, *trans*-nonachlor was detected in nearly in all subjects, with a peak level of 4.5 ppb. However, little *cis*- or *trans*-chlordane, which were primary components of the termiticide, was detected, and oxychlordane and heptachlorepoxide, which were not contained in the termiticide, were detected at high concentrations with peaks of 4.37 ppb and 13.07 ppb, respectively. Of the 8 subjects, the blood chlordane levels were highest (21.81 ppb as the total chlordane concentration) in F, who had been engaged in termite control work for 20 years before the ban of the use of Chlordane.

Table 1 Blood chlordane concentrations of termite control workers in 1987

Worker	Period of engagement	Compounds (ppb) 1	2	3	4	5	6	7
A	1 year	N.D.	N.D.	0.42	0.68	N.D.	N.D.	N.D.
B	7 years	N.D.	N.D.	1.27	3.02	N.D.	N.D.	N.D.
C	1 year	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
D	2 years	N.D.	0.85	2.36	3.23	N.D.	N.D.	N.D.
E	4 years	N.D.	1.34	2.16	4.93	0.37	N.D.	0.89
F	20 years	N.D.	4.37	4.44	13.07	N.D.	0.22	1.08
G	3 years	N.D.	0.50	0.59	0.48	N.D.	N.D.	N.D.
H	12 years	N.D.	1.35	4.55	1.36	N.D.	N.D.	0.27

N.D. : Not detected at the detection limit of 0.2ppb in blood

1: heptachlor, 2: oxychlordane, 3: *trans*-nonachlor, 4: heptachlorepoxide, 5: *trans*-chlordane, 6: *cis*-chlordane, 7: *cis*-nonachlor

Next, the relationships between the blood chlordane concentrations and the period of engagement in termite control before the ban were examined. A strong positive correlation with a correlation coefficient (r) of 0.876 ($p<0.01$) was observed for the total-chlordane concentration (Fig.2). The concentrations of *trans*-nonachlor ($r=0.843$, $p<0.01$), oxychlordane ($r=0.876$, $p<0.01$), and heptachlorepoxide ($r=0.802$, $p<0.05$) also showed strong positive correlations with the number of years in the work, indicating that they were higher as the worker was engaged in the work longer before the ban. Therefore, the blood chlordane concentrations are considered to reflect the extent of past exposure, and *trans*-nonachlor, oxychlordane, and heptachlorepoxide to be especially useful as indices for biological monitoring.

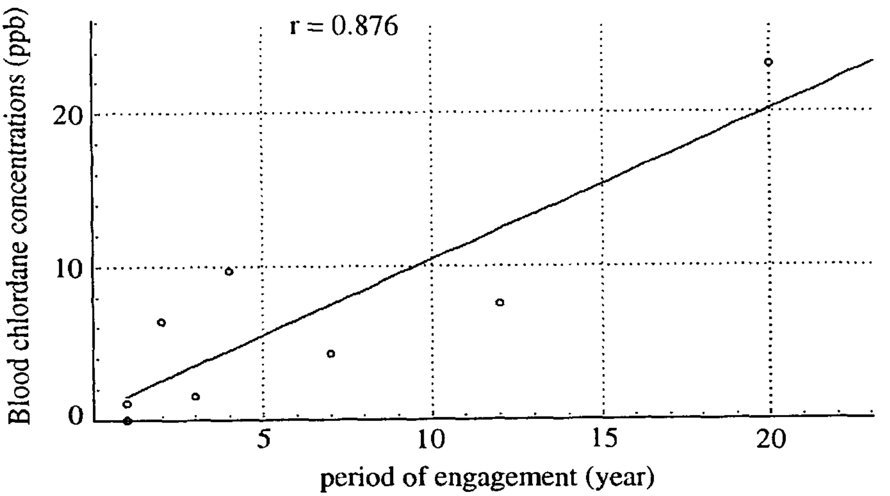

Figure 2 Relationships between the blood (1987) chlordane concentrations (total of 7 compounds) and the period of engagement in termite control before the ban (1986) of Chlordane using

Fig. 3 shows changes in the blood chlordane concentrations in the termite control workers after discontinuation of the use of Chlordane. In general, they tended to decrease with time. The mean clordane concentrations in the 8 workers was 6.73 ppb 1 year after discontinuation, but it decreased to 4.95 ppb after 4 years. However, the percent decrease was only 26%, and the decrease was not clear in F, who was exposed over a long period and showed high blood concentrations. In individual workers, the percent decrease in the chlordane concentration also showed a strong negative correlation ($r=-0.919$, $p<0.01$) with the period in the work before the ban. Therefore, the decrease in the blood chlordane concentrations were slower as a worker was engaged in the work longer before the ban of the use of Chlordane, i.e. as he was exposed to a greater extent.

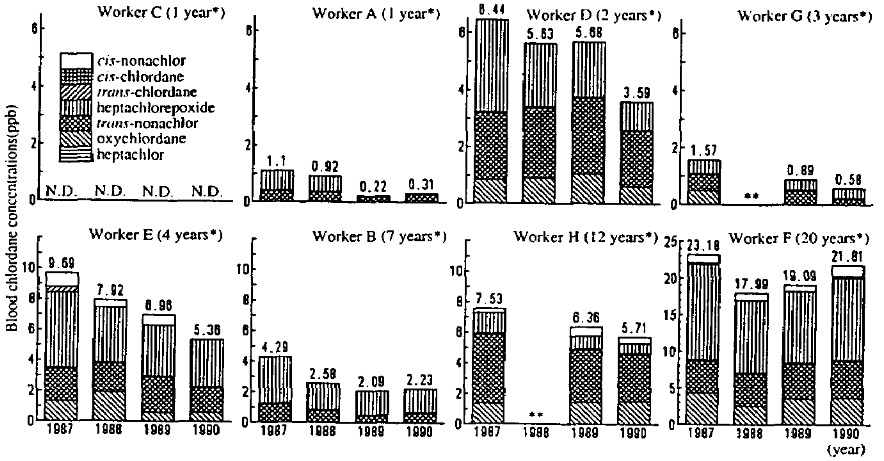

Figure 3 Changes in the blood chlordane concetrations in the termite control workers after discontinuation of Chlordane using

N.D.: Not detected at the detection limit of 0.2ppb in blood

* Period of engagement in termite control before the ban (1986) of Chlordane using

**No sample

Of the 7 compounds, heptachlorepoxide showed a large percent decrease, oxychlordane also showed a relatively large percent decrease, but that of *trans*-nonachlor was small.

At any rate, chlordanes are suggested to persist at considerable concentrations in the bodies of termite control workers even several years after discontinuation of the use of Chlordane, and the residual chlordane levels to be associated with the extent of exposure.

According to earlier reports, the mean blood total-chlordane concentrations in Japanese termite control operators or pest control operators were 16.1 ppb (Kawano and Tatsukawa, 1982) and 0.89 ppb (Saito et al., 1986). The mean blood total-chlordane concentration in individuals without occupational exposure to chlordane was reported to be about 0.5 ppb (Wariishi et al., 1986). In the 8 termite control workers evaluated in this study, high levels of chlordanes were observed, and the total-chlordane concentration 1 year after discontinuation of the use of Chlordane was 23.18 ppb at the maximum (6.73 ppb as the mean of the 8 subjects). Although the amount of chlordane handled by these workers in the past or details of the contents of the work are unknown, the blood chlordane concentrations were higher as the period of engagement in the operation was longer. Since organic chloride agricultural chemicals have a strong tendency to be deposited in fat, and their concentrations in the adipose tissue are reported to be 100-10,000 times higher than their blood concentrations (Sasaki et al., 1991; Mes, 1992), large amounts of chlordanes are considered to be deposited in the bodies of these termite control operators. Also, measurement of the blood chlordane concentrations is suggested to be useful for biological monitoring.

Trans-nonachlor, oxychlordane, and heptachlorepoxide were detected at higher levels than other chlordanes in blood. *Cis*- and *trans*-chlordane are primary components of the termiticide, being contained at 25% and 20%, respectively. Of the more than 20 compounds contained as contaminants, heptachlor accounted for about 10%, *trans*-nonachlor for 7%, and *cis*-nonachlor for a few percent. Concerning the metabolism of Chlordane, Tashiro and Matsumura (1977) observed that *trans*-chlordane is metabolized primarily via oxychlordane, but *cis*-chlordane is metabolized also by direct hydroxylation reaction. In any case, oxychlordane is a primary metabolite of chlordane. Heptachlor is metabolized mainly via heptachlorepoxide (Tashiro and Matsumura, 1978). Probably for this reason, little *trans*-chlordane or *cis*-chlordane, which were primary components of the termiticide, was detected in blood, and heptachlor, which accounted for a relatively high percentage among contaminants, was absent, but oxychlordane and heptachlorepoxide were detected at high levels. Among the compounds contained in the termiticide, *trans*-nonachlor was detected at a high level. It is reported to be dechlorinated first to *trans*-chlordane and then to be metabolized by the above metabolic pathway of *trans*-chlordane (Tashiro and Matsumura, 1978). However, it occupied 35% (mean of 8 workers) 1 year after discontinuation of the use of Chlordane and 50% after 4 years. These percentages are strikingly high as compared with its contents in the original termiticide, which was only 7%. This suggests that *trans*-nonachlor is more resistant to metabolism than *trans*-chlordane, *cis*-chlordane, or heptachlor. Also, *trans*-nonachlor showed a low percent decrease, suggesting that it has a greater tendency to persist in the body than other compounds observed at high levels in blood such as heptachlorepoxide and oxychlordane.

We analyzed blood of termite control workers using the wide bore capillary column gas chromatography, which we developed for simultaneous analysis of blood chlordanes. The blood levels of chlordanes generally decreased after discontinuation of the use of Chlordane, but *trans*-nonachlor, oxychlordane, and heptachlorepoxide were detected at high levels even after several years. These findings suggest that chlordanes are deposited in large amounts in the bodies of termite control workers, and that biological monitoring (measurement of blood chlordane levels) by this method is important. *Trans*-nonachlor, oxychlordane, and heptachlorepoxide are considered to be useful as indices for this biological monitoring.

REFERENCES

Kawano M, Tatsukawa R (1982) Chlordanes and related compounds in blood of pest control operators (pcos). J Agric Chem Soc Jpn, 56: 923-929.

Miyazaki T, Akiyama K, Kaneko S, Horii S, Yamagishi T (1980a) Identification of chlordane and related compounds in goby-fish from Tokyo bay. Bull Environ Contam Toxicol, 24: 1-8.

Miyazaki T, Akiyama K, Kaneko S, Horii S, Yamagishi T (1980b) Chlordane residues in human milk. Bull Environ Contam Toxicol, 25: 518-523.

Mes J (1992) Organochlorine residues in human blood and biopsy fat and their relationship. Bull Environ Contam Toxicol, 48: 815-820.

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 Hlth, 58: 91-97.

Sapp R E (1989) Recoveries of chlorinated hydrocarbon pesticides from fat using florisil and sep-paks. J Agric Fd Chem, 37: 1313-1317.

Sasaki K, Ishizaka T, Suzuki T, Takeda M, Uchiyama M (1991) Accumulation levels of organochlorine pesticides in human adipose tissue and blood. Bull Environ Contam Toxicol, 46: 662-669.

Tashiro S, Matsumura F (1977) Metabolic routes of *cis*- and *trans*-chlordane in rats. J Agric Fd Chem, 25: 872-880.

Tashiro S, Mastumura F (1978) Metabolism of *trans*-nonachlor and related chlordane components in rat and man. Arch Environ Contam, 7: 113-127.

Takeda M, Sekita H, Sasaki K, Kawamura Y, Uchiyama M, Takahashi A, Ohno Y, Kawanishi T, Takanaka A (1984) Distribution of chlordane components in rat tissues after daily oral administration of technical chlordane at low levels. Bull National Institute of Hygienic Science, 102: 113-117.

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

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

Yamagishi T, Miyazaki T, Akiyama K, Kaneko S, Horii S (1981) Residues of chlordanes in fish and shellfish from kanto area and its vicinity. J Fd Hyg Soc Jpn, 22: 270-278.