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Accumulation of Chlordanes in Adipose Tissues of Mice Caused by Long-Term Exposure of Low Level Technical Chlordane

F. Asakawa,[1,2] F. Jitsunari,[2] H. Shiraishi,[2] S. Suna,[2] N. Takeda,[2] T. Kitamado[2]

[1]Human-Environmental Science Research Laboratory, College of Liberal Arts and Science, Kurashiki University of Science and the Arts, 2640 Nishinoura, Tsurajima-cho, Kurashiki-shi, Okayama 712, Japan
[2]Department of Hygiene and Public Health, Kagawa Medical School, 1750-1 Ikenobe, Miki-cho, Kagawa 761-07, Japan

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Chlordane is an organochlorine pesticide (termiticide), which was used mainly for termite control in Japan, Since its chronic toxicity (causing liver dysfunction) as well as its decomposability and accumulation were recognized, its use has been prohibited by a regulation established in September, 1986. The technical chlordane (Chlordane) is a mixture of more than 20 compounds, consisting of *trans*-chlordane (25%) *cis*-chlordane (20%) heptachlor (10%) *trans*-nonachlor (7%) and *cis*-nonachlor (< 5%).

In Japan, contamination of the sediment and fish with Chlordane has been reported (Miyazaki et al., 1980, Yamagishi et al., 1981). We have shown that indoor air is contaminated by vaporization of residual Chlordane in houses (Jitsunari et al., 1987b) which persists long time with seasonal variation becoming high-level in summer (Asakawa et al., 1994). It has also shown that the level of chlordane compounds/metabolites (chlordanes) in the skin and blood of the residents of houses treated with Chlordane is higher than that of the residents in non-treated houses (Hirai and Tomokuni, 1993) indicating the risk of indoor air contamination with Chlordane. However, the effects of prolonged inhalation of indoor air contaminated by a low level of Chlordane have not been examined.

In this study, we performed exposure tests of indoor air contamination using mice, and found a high level of *trans*-nonachlor, oxychlordane and heptachlorepoxide in the adipose tissues of mice and a dose-effect relationship.

MATERIALS AND METHODS

A group of 5 mice aged 8 weeks (Std, ddy ♀) in a cage were placed in an exposure chamber, and exposed to Chlordane by placing a piece of felt (ϕ 60 mm) impregnated with 2 mL of 2% Chlordane at the indraft opening of the chamber (indraft, 75 L/min). The size of the chamber was 342 (width) x 412 (depth) x 286 (height) mm, and the cage was 215 (width) x 320 (depth) x 130 (height) mim.

Correspondence to: F. Asakawa

As shown in Table 1, exposure periods were about l-6 months, and the Chlordane level in the chamber was monthly monitored by the diffusion sampler method as reported previously (Jitsunari et al., 1987a). Gas chromatography-mass spectrometry (GCMS) was used for quantification of Chlordane. As controls, 2 groups of mice were kept for 6 months without exposure to Chlordane. To analyze chlordanes in the adipose tissues, 3 of the 5 mice in each group were used.

During the test, the mice were kept under standard conditions (temperature, 24 ± 2 °C; humidity, 50-70%; continuous feeding; bottle watering) in the animal experiments laboratory of our university. This test had been approved by the animal experimentation committee of the university, and was carried out according to the animal experimentation guidelines.

To measure chlordanes in the adipose tissues, we developed a simple and rapid method using solid phase extraction for sample pretreatment. Two grams of the adipose tissue sample was extracted with 100 mL mixture of acetone : hexane = 1 : 2 (extraction solvent) by high-speed homogenization (1 min) using a biotron (IKA-ULTRA-TURRX T25) and sonication (5 min). The extraction was left standing for a while, and the supernatant was subjected to suction filtration. The residue was reextracted with 30 mL of the extraction solvent, sonicated for 1 min, and subjected to suction filtration. This procedure was repeated three times, and all filtrates were combined. The filtrate obtained was concentrated using a rotary evaporator in a water bath at a temperature not higher than 40 °C under reduced pressure until the solvent was completely removed. The residue was then dissolved in 2 mL of hexane, and placed on an extrelute column (Merck, Extrelute-3). After the sample was allowed to spread on the column for 20 min, chlordanes were eluted in 30 mL of acetonitrile. The solution was concentrated in a water bath at a temperature not higher than 40 °C under reduced pressure until the solvent was completely removed using a rotary evaporator, and dissolved in 1 mL of hexane. To clean up the sample for GCMS analysis, it was injected into a Sep-pak florisil (Waters) that had been prewashed with 10 mL of hexane, and Chlordanes were eluted in 10 mL of hexane containing 1% ether. The eluate was concentrated under reduced pressure using a Kuderna-Danish evaporative concentrator, dissolved in hexane to 1 mL exact, and subjected to GCMS.

Chlordanes examined were the following 7 compounds; *cis*-, *trans*-chlordane which are the main constituents of technical chlordane, heptachlor, *cis*-, *trans*-nonachlor, which are secondary constituents, and oxychlordane and heptachlorepoxide, which are the main metabolites (Tashiro and Matsumura, 1977 and 1978). Measurements were performed by the selected ion monitoring method (SIM), using a GCMS (Shimadzu GCMS-QP2000GF). The measurement conditions are as follows. Column; CBJ-S30-0.25 (Shimadzu), 30 m x 0. 32 mm I.D., 0.25 μm, Carrier; He 10 mL/min, Oven; 120-200 °C at 10 °C/min, 200-230 °C at 5 °C /min, 230-310 °C at 10 °C/min, Injection; 250 °C, m/z: heptachlor; 272 274, heptachlorepoxide; 353 355, *cis*-, *trans*-chlordane; 373 375, oxychlordane; 387 389, *cis*-, *trans*-nonachlor; 407 409.

Reagents used were of the grade for residual pesticide analysis or equivalents purchased from the suppliers, As standards, heptachlor, *cis*-, *trans*-chlordane and heptachlorepoxide purchased from Wako Pure Chemical Industries and oxychlordane and *cis*-, *trans*-nonachlor purchased from Nanogen Inc. were used. Standard solutions were prepared by dissolving standard samples in hexane.

RESULTS AND DISCUSSION

Table 1 shows the exposure conditions. The mean exposure level (mean Chlordane level in the air) was 4.22-11.36 μg/m^3 (total of 5 compounds). *Trans*-chlordane and *cis*-chlordane were present at high levels, 38% and 31% of the total chlordanes on average, respectively. Heptachlor and *trans*-nonachlor accounted for 15% and 14%, respectively, while *cis*-nonachlor showed a low percentage of 2%.

Table 2 shows the body weights of the mice. There was no significant difference between the exposed and non-exposed groups.

Chlordanes in adipose tissues are conventionally analyzed by repeated solution-solution extraction of samples that have been extracted in an organic solvent. However, this method has disadvantages requiring a large amount of extraction solvents, being time-consuming, and involving complicated procedures. Recently, the solid phase extraction method has become the focus of interest as a useful method for chemical separation and purification. We applied this method to the analysis of chlordanes and attempted to reduce the amount of organic solvents used and to establish a rapid and simple method of analysis. SIM chromatograms of the standard solutions of chlordanes (7 compounds) and of the sample prepared by solid phase extraction of the mixture of adipose tissue (non-exposed) and the standard solutions are shown in Fig. 1. Here, SIM chromatograms at one m/z of each constituent are shown. There are no obstructing peaks of impurities in the adipose tissue samples, and complete separation of the 7 compounds was obtained. This result indicated that the solid phase extraction method combined with the SIM method would be appropriate for the analysis of chlordanes in adipose tissues. The recovery rate was nearly 100%. In this study, we applied nonaqueous samples on an Extrelute-3 column, and successfully separated chlordanes from adipose tissues by eluting with a hydrophilic organic solvent (acetonitrile). By this solid phase extraction method, the amount of the solvent used was reduced to about 1/6-1/7 of the solvent used in the conventional solution-solution extraction method, and the time for analyses was shortened to about 1/2, showing that this method is much simpler and quicker. Care must be taken that samples are not eluted out the column immediately after the application of the sample. In the Extrelute-3 column used in this study, 3 mL was the maximum that can be applied. As shown in Table 3, 4.19-10.63 ppm of chlordanes (total of the 7 compounds) were present in adipose tissue. Among the constituents of the original chlordanes, heptachlor and *trans*-chlordane were below the detection limit (< 0.005 ppm), and

Table 1. Exposure condition of technical chlordane to mice.

Group (n=5)	Exposure day	Exposure concentration (μg/m³)				
		Hepta[1]	*t*-Chl[2]	*c*-Chl[3]	*t*-Nona[4]	*c*-Nona[5]
A	32	1.77	4.29	3.57	1.59	0.14
B	32	1.44	3.80	3.19	1.43	0.15
C	62	1.66	3.55	2.73	1.22	0.09
D	62	1.83	4.15	3.32	1.44	0.12
E	126	0.64	1.94	1.62	0.78	0.12
F	126	1.01	2.89	2.38	1.12	0.13
G	181	0.53	1.61	1.33	0.65	0.10
H	181	0.74	2.12	1.75	0.82	0.11

As controls, 2 groups of mice were kept for 181 days without exposure to Chlordane.
1. heptachlor, 2. *trans*-chlordane, 3. *cis*-chlordane, 4. *trans*-nonachlor, 5. *cis*-nonachlor

Table 2. Body weights (mean, g) of mice.

Exposed	days	0	7	14	21	32	62	126	181
Control		28.6	31.0	32.0	33.7	35.1	37.6	42.9	46.9
(n)		(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Exposure		28.2	31.0	32.2	34.0	36.1	39.4	44.6	48.5
(n)		(40)	(40)	(40)	(40)	(40)	(30)	(20)	(10)

No significant difference between the exposed and non-exposed (control) groups.

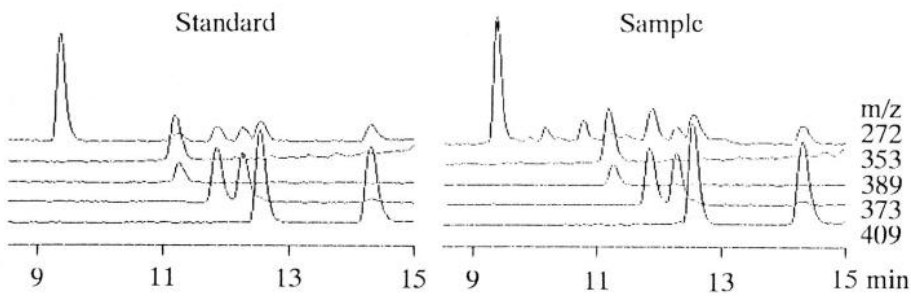

Figure 1. SIM chromatograms of chlordanes standard solution and of sample prepared by solid phase extraction of the mixture of adipose tissue (non-exposed) and the standard solution.

m/z: 272; heptachlor, 353; heptachlorepoxide, 389; oxychlordane, 373; *trans*-chlordane, *cis*-chlordane (later peak), 409; *trans*-nonachlor, *cis*-nonachlor (later peak)

Table 3. Accumulation of chlordanes in adipose tissues of mice caused by long-term exposure of low level technical chlordane.

Group	Compounds (mean, ppm)						
(n=3)	Hepta[1]	Epoxide[2]	Oxy[3]	*t*-Chl[4]	*c*-Chl[5]	*t*-Nona[6]	*c*-Nona[7]
Cont.1	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Cont.2	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
A	N.D.	1.30	1.30	N.D.	0.11	3.22	0.15
B	N.D.	0.94	0.86	N.D.	0.06	2.31	0.12
C	N.D.	1.38	1.21	N.D.	0.08	2.48	0.09
D	N.D.	1.47	1.47	N.D.	0.09	3.67	0.14
E	N.D.	0.75	1.20	N.D.	0.05	2.09	0.10
F	N.D.	2.49	3.04	N.D.	0.13	4.79	0.18
G	N.D.	1.72	2.44	N.D.	0.13	3.94	0.19
H	N.D.	1.75	2.15	N.D.	0.12	3.43	0.17

N.D.: Below the detection limit (< 0.005 ppn) in adipose tissue.
1. heptachlor, 2. heptachlorepoxide, 3. osychlordane, 4. *trans*-chlordanc, 5. *cis*-chlordane 6. *trans*-nonachlor, 7. *cis*-nonnchlor

cis-chlordane was negligibly detected. On the other hand, *trans*-nonachlor was present at 2.09-4.79 ppm, and oxychlordane and heptachlorepoxide, which are metabolites of the original compounds, were detected at levels of 0.86-3.04 ppm and 0.75-2.49 ppm, respectively. Relative abundance of the 7 compounds in the adipose tissue differed significantly from that in the air. *Trans*-nonachlor accounted for about 50% on average, a very high proportion compared to its level in the air. On the other hand, *trans*-chlordane, which was present at a high level in the air, was not detected in the adipose tissue and the proportion of *cis*-chlordane was only 1%. Heptachlor was not detected, while the proportion of *cis*-nonachlor in the adipose tissue was 2%, equivalent to its proportion in the air. The proportion of oxychlordane and heptachlorepoxide was 25% and 22%, respectively. Those results show that *trans*-nonachlor, oxychlordane and heptachlorepoxide accumulate in the adipose tissue of mice when exposed to chlordanes at the levels of contaminated indoor air. The proportion of *trans*-nonachlor in the adipose tissue showed a tendency to decrease with increasing time of exposure (53 $\rightarrow$ 51 $\rightarrow$ 48 $\rightarrow$ 46%), while that of oxychlordane increased (21 $\rightarrow$ 22 $\rightarrow$ 29 $\rightarrow$ 29%). No chlordanes were detected in the mice before exposure (2 groups) and in the mice kept without exposure for 6 months (2 groups).

The dose-effect relationship is shown in Fig. 2. The abscissa shows the exposure amount of Chlordane, dose (the total level of the 5 compounds in the air x exposure period , $\mu g/m^3$ x day), and the ordinate is the level of chlordanes in the adipose

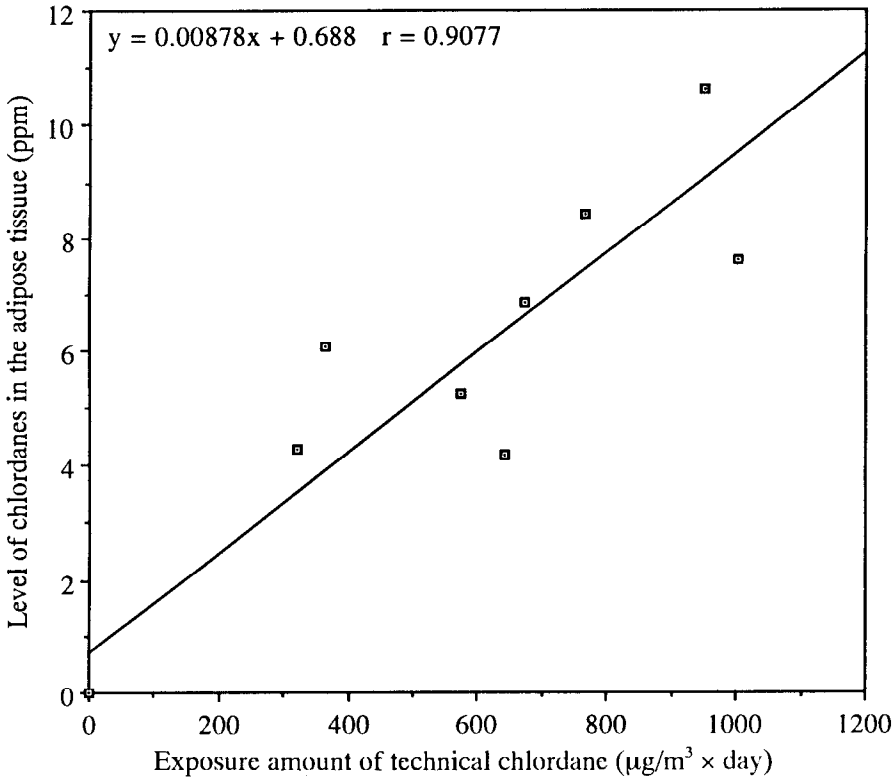

Figure 2. Dose-effect relationship between the exposure amount of technical chlordane (μg/m³ x day) and the level of chlordanes in the adipose tissuue (ppm).
The abscissa is the total level of the 5 compounds in the air x exposure period.
The ordinate is the total level of the 7 compounds in the adipose tissue.

tissue (the total level of the 7 compounds, ppm). The results for the 2 non-exposure groups (dose=0, chlordanes level in the adipose tissue=0) are also included in the graph. There was a significant correlation between these variables, with r=0.9077 (n=10, $p < 0.01$) indicating that the level of chlordanes in the adipose depends on the exposure amount of Chlordane. The regression line was expressed as y=0.00878x + 0.688.

In our previous survey (Jitsunari et al., 1987b), the maximum level of Chlordane in the indoor air was 3.37 μg/m³. The surveys performed in the USA showed that most of Chlordane levels of indoor air were below 10 μg/m³ (Livingston and Jones, 1981; Wright and Leidy, 1982; Leidy and Wright, 1985). Therefore, the Chlordane level used in the exposure test in this study (4.22-11.36 μg/m³) was similar to that of indoor air contaminated with Chlordane. The body weight of the mice exposed to chlordane at these levels over a period of 1-6 months was not

different from the non-exposed mice. However, 4.19-10.63 ppm of chlordanes (total of the 7 compounds) were accumulated in the adipose tissue. The main compounds of the accumulated chlordanes were *trans*-nonachlor (about 50%) oxychlordane (about 25%) and heptachlorepoxide (about 22%) which together accounted for nearly 100% of the accumulated chlordanes. On the other hand, *trans*-chlordane, *cis*-chlordane and heptachlor, which are constituents of technical chlordane and present at high levels in the air, were negligibly detected in the adipose tissue. Takeda et al. (1984) found that in rats orally administered with 10 µg/kg/day chlordane using a stomach probe for 1 and 2 weeks, oxychlordane, *trans*-nonachlor and heptachlorepoxide were significantly accumulated in the adipose tissue, and only trace amounts of *trans*-chlordane and *cis*-chlordane, the main constituents of chlordane, were found in the adipose tissue after 2 weeks. Satoh et al. (1992) reported that when mice were orally administered with a single dose of a mixture of *trans*-chlordane and *cis*-chlordane, they rapidly disappeared from the tissues, but oxychlordane remained in several tissues even at 52 weeks after administration.

The study of chlordane metabolism in animals by Tashiro and Matsumura (1977) revealed that *trans*-chlordane is mainly metabolized via oxychlordane, and that *cis*-chlordane is metabolized via oxychlordane and by direct hydroxylation reaction, indicating that oxychlordane is the main metabolite of Chlordane. Heptachlor is mainly metabolized via heptachlorepoxide (Tashiro and Matsumura, 1978). Therefore, it is reasonable that *trans*-chlordane and *cis*-chlordane, the main constituents of chlordane, and heptachlor, present at a high proportion in chlordane, were negligibly detected in the tissue, while oxychlordane, the major metabolite of *trans*-chlordane and *cis*-chlordane, and heptachloreoxide, the major metabolite of heptachlor, were detected at high levels. On the other hand, *trans*-nonachlor, which account for only about 14% of chlordanes in the air (the fourth largest amount), was found to be about 50% of the chlordanes in the adipose tissue (the largest amount). *Trans*-nonachlor is dechlorinated to become *trans*-chlordane, and metabolized in the metabolic pathway described above (Tashiro and Matsymura, 1978). However, our findings suggest that *trans*-nonachlor is not readily metabolized. With prolonged exposure, we observed a gradual decrease in the proportion of *trans*-nonachlor and an increase in the proportion of oxychlordane in the adipose tissue, suggesting that *trans*-nonachlor is gradually metabolized.

In the long-term exposure tests at a level of chlordane in the indoor air using mice, *trans*-nonachlor, oxychlordane and heptachlorepoxide were accumulated in the adipose tissue of the mice, and a dose-effect relationship was observed. It is suggested that even at a low level of chlordane in indoor air, chlordanes are steadily accumulated in the adipose tissue of residents with prolonged exposure. Therefore, it is necessary to investigate risks caused by chlordane contamination of indoor air.

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