Tissue Concentrations of Chlordanes in Mice after Long-term Exposures to Technical Grade Chlordane at Indoor Air Levels

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Abstract

We determined concentrations of chlordanes (5 chlordane compounds and 2 metabolites) in tissues and organs of mice following a prolonged exposure to Chlordane (technical grade chlordane) at levels as low as those in indoor air.

After exposure to 4.22-11.36 $\mu\text{g}/\text{m}^3$ Chlordane (total of 5 compounds) in the air for 1-6 months, 6.44-13.00 ppm chlordanes (total of 7 compounds) were detected in mice (2 tissues and 6 organs). The adipose tissue among the 2 tissues / 6 organs examined contained the highest chlordanes. The ratio of the adipose tissue chlordanes to the liver chlordanes was approximately 5.6 times, followed by the muscle (0.9), lungs (0.4), kidneys (0.4), heart (0.3), spleen (0.2) and brain (0.1). In addition, *trans*-nonachlor, oxychlordane and heptachlorepoxyde in chlordanes were at a high level.

The level of Chlordane exposure (dose) and the level of chlordanes accumulation in each tissue or organ of the mice except for the heart were closely correlated ($r=0.9388-0.7130$), and showed a linear relationship. The tendency of chlordanes accumulation in light of the linear relationship was adipose $\gg$ liver $\geq$ muscle.

Thus, even with a low level of Chlordane in indoor air, chlordanes may be steadily accumulated in the bodies of human residents with prolonged exposure similarly.

The present findings suggest that it is necessary to investigate the risk of organochlorine chemicals contamination in indoor air at prolonged exposure.

Key words: Chlordane, Indoor air contamination, Long-term, Exposure, Accumulation

Introduction

Cases of indoor air contamination by chemicals have increased recently in Japan¹⁻⁴⁾. This may be due to the improved airtightness of houses, and / or due to the increased use of chemicals indoor.

Indoor air contamination can be caused by several chemicals while organochlorine chemicals are just one of the examples. We have shown that indoor air has been contaminated by the vaporization of residual Chlordane⁵⁾, an organochlorine pesticide (termiticide) which has been used mainly for termite control in Japan. It has also been shown that the amounts of chlordane

compounds and metabolites (chlordanes) present on the skin and in the blood of residents of houses treated with Chlordane are higher than that of the residents in non-treated houses⁶⁾. Owing to Chlordane's chronic toxicity (liver dysfunction) as well as its accumulation and low decomposability, its use in Japan has been prohibited since September, 1986. However, we have shown that residual Chlordane in houses persists a long time⁷⁾ and shows seasonal variations, with the highest level in summer⁸⁾.

Therefore, in residential environments in which Chlordane was used, the effect of the exposure of the residents to the vapor of residual Chlordane may be long term, and it is necessary to investigate the risks presented by the Chlordane contamination of indoor air. However, to our knowledge, examination of the accumulation after prolonged inhalation of indoor air contaminated by low levels of Chlordane has not been conducted.

In the present study, the concentrations of chlordanes (5 chlordane compounds and 2 metabolites) in tissues and organs of

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mice following a prolonged exposure to Chlordane (technical grade chlordane) at levels as low as those in indoor air was determined with the aim of obtaining basic information concerning accumulation of chlordanes in the bodies of human residents.

Materials and Methods

Female ddy mice aged 8 weeks were raised together in groups of 5 per cage maintained in an exposure chamber, and were exposed to vaporizing technical chlordane (Chlordane) by the placement of 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). Chlordane used as a termiticide is a mixture of more than 20 compounds: its approximate constitution is *trans*-chlordane (25 %), *cis*-chlordane (20 %), heptachlor (10 %), *trans*-nonachlor (7 %) and *cis*-nonachlor (< 5 %). The size of the chamber was 342 (width) $\times$ 412 (depth) $\times$ 286 (height) mm, and the cage was 215 (width) $\times$ 320 (depth) $\times$ 130 (height) mm.

Table 1 shows the exposure concentrations of chlordane and impurities. The exposure periods were approximately 1-6 months. The Chlordane level in the chamber was monitored monthly by the diffusion sampler method as reported previously^{9,10}. Gas chromatograph-mass spectrometry (GCMS) was used for the quantification of Chlordane. The mean Chlordane exposure level was 4.22-11.36 $\mu\text{g}/\text{m}^3$ (the total of 5 compounds). Among the five compounds of Chlordane, *trans*-chlordane and *cis*-chlordane were present at the highest levels (38 % and 31 %, respectively). Heptachlor and *trans*-nonachlor accounted for 15 % and 14 %, respectively, while *cis*-nonachlor showed a low percentage, 2 %. As controls, 2 groups of mice ($n = 5$ each) were maintained for 6 months without exposure to Chlordane. During the experiment, all mice were maintained under standard conditions (temperature, 24 $\pm$ 2 °C; humidity, 50-70 %; continuous feeding; bottle watering) in the animal laboratory of Kagawa Medical University. This experiment was approved by the Animal Experimentation Committee of the University, and was carried out according to the animal experimentation guidelines.

For the analysis of chlordanes in the mice, samples of 2 tissues and 6 organs were collected from 3 of the 5 mice in each group at the end of each exposure period. These samples were the adipose (abdomen), muscle (thigh) tissues, and the brain, lungs, heart, liver, kidneys and spleen. These 3 mice in each group were random sampling while the remaining 2 were used for another study.

To measure chlordanes in the adipose tissues, we used a simple and rapid solid phase extraction method for sample pretreatment as reported previously¹¹. The adipose tissue sample (≤ 2 g) was extracted with a 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 re-extracted 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 (Extrelute-3, Merck). 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 then dissolved in 1 mL of hexane. To clean up the sample for the GCMS analysis, it was injected into a Sep-pak florasil (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, and dissolved in hexane to 1 mL exact, and subjected to GCMS. The same method was also used for the other tissue and organ samples; however, it was possible to omit the Extrelute column extraction procedure (except for the analysis of the brain samples).

The number of samples used for the measurement of chlordanes in the adipose, muscle and liver was 3 (each mouse), whereas for the other organs, the samples from 3 mice were mixed together because of the small amounts.

The following 7 chlordane compounds were measured: *cis*-chlordane and *trans*-chlordane (which are the main constituents of Chlordane), heptachlor, *cis*-nonachlor and *trans*-nonachlor (secondary constituents), and oxychlordane and heptachlorepoide, which are the main metabolites. Measurements were performed by the selected ion monitoring method (SIM), using a GCMS (GCMS-QP2000GF, Shimadzu). The measurement conditions were as follows: column, CBJ-S30-0.25 (Shimadzu), 30 m $\times$ 0.32 mm I.D., 0.25 μm film thickness; 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 and 274, heptachlorepoide: 353

Table 1 Exposure concentrations of chlordane and impurities.

Group (sampler)	Number of days of exposure	Exposure concentration ($\mu\text{g}/\text{m}^3$) per day						Dose ⁷ ($\mu\text{g}/\text{m}^3 \times \text{day}$)
		Hepta ¹	<i>t</i> -Chl ²	<i>c</i> -Chl ³	<i>t</i> -Nona ⁴	<i>c</i> -Nona ⁵	Total ⁶	
A (n=1)	32	1.77	4.29	3.57	1.59	0.14	11.36	363.52
B (n=1)	32	1.44	3.80	3.19	1.43	0.15	10.01	320.32
C (n=2)	62	1.66 $\pm$ 0.33	3.55 $\pm$ 0.66	2.73 $\pm$ 0.63	1.22 $\pm$ 0.20	0.09 $\pm$ 0.01	9.25	573.50
D (n=2)	62	1.83 $\pm$ 0.63	4.15 $\pm$ 0.60	3.32 $\pm$ 0.32	1.44 $\pm$ 0.13	0.12 $\pm$ 0.01	10.86	673.32
E (n=4)	126	0.64 $\pm$ 0.30	1.94 $\pm$ 1.14	1.62 $\pm$ 1.00	0.78 $\pm$ 0.50	0.12 $\pm$ 0.08	5.10	642.60
F (n=4)	126	1.01 $\pm$ 0.22	2.89 $\pm$ 0.56	2.38 $\pm$ 0.43	1.12 $\pm$ 0.18	0.13 $\pm$ 0.03	7.53	948.78
G (n=6)	181	0.53 $\pm$ 0.12	1.61 $\pm$ 0.41	1.33 $\pm$ 0.37	0.65 $\pm$ 0.20	0.10 $\pm$ 0.05	4.22	763.82
H (n=6)	181	0.74 $\pm$ 0.21	2.12 $\pm$ 0.98	1.75 $\pm$ 0.87	0.82 $\pm$ 0.44	0.11 $\pm$ 0.08	5.54	1002.74

As controls, 2 groups of mice were maintained for 181 days without exposure to Chlordane.

¹ heptachlor, ² *trans*-chlordane, ³ *cis*-chlordane, ⁴ *trans*-nonachlor, ⁵ *cis*-nonachlor, ⁶ mean of 5 compounds, ⁷ Total $\times$ day

and 355, *cis*-chlordane: 373 and 375, *trans*-chlordane: 373 and 375, oxychlordane: 387 and 389, *cis*-nonachlor: 407 and 409, *trans*-nonachlor: 407 and 409.

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

Results

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

Table 3 shows examples of the chlordanes (7 compounds) analysis in the mice exposed to Chlordane; the periods of 1 month (Group A and Group B) and 6 months (Group G and Group H). No chlordanes were detected in the mice before exposure (2 groups) or in the mice maintained without exposure

Table 2 Body weights (mean $\pm$ SD, g) of mice.

Exposed days	0	7	14	21	32	62	126	181
Control	28.6 $\pm$ 1.5	31.0 $\pm$ 1.6	32.0 $\pm$ 1.5	33.7 $\pm$ 1.4	35.1 $\pm$ 1.4	37.6 $\pm$ 2.7	42.9 $\pm$ 4.0	46.9 $\pm$ 5.0
(n)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Exposure	28.2 $\pm$ 1.3	31.0 $\pm$ 1.7	32.2 $\pm$ 2.4	34.0 $\pm$ 2.3	36.1 $\pm$ 2.7	39.4 $\pm$ 3.9	44.6 $\pm$ 6.2	48.5 $\pm$ 8.6
(n)	(40)	(40)	(40)	(40)	(40)	(30)	(20)	(10)

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

Table 3 Example of the chlordanes analysis in the mice exposed to technical chlordane; periods of 1 month (Group A and Group B) and 6 months (Group G and Group H).

	Hepta ¹ (ppm)	Epoxide ² (ppm)	Oxy ³ (ppm)	<i>t</i> -Chl ⁴ (ppm)	<i>c</i> -Chl ⁵ (ppm)	<i>t</i> -Nona ⁶ (ppm)	<i>c</i> -Nona ⁷ (ppm)	Total ⁸ (ppm)
Group A								
Adipose(n=3)	ND	1.30 $\pm$ 0.03	1.30 $\pm$ 0.19	ND	0.11 $\pm$ 0.03	3.22 $\pm$ 0.47	0.15 $\pm$ 0.04	6.08
Liver (n=3)	ND	0.24 $\pm$ 0.04	0.22 $\pm$ 0.06	0.05 $\pm$ 0.01	0.01 $\pm$ 0.00	0.56 $\pm$ 0.08	0.04 $\pm$ 0.01	1.12
Muscle(n=3)	ND	0.13 $\pm$ 0.06	0.30 $\pm$ 0.15	0.05 $\pm$ 0.03	0.01 $\pm$ 0.00	0.65 $\pm$ 0.34	0.03 $\pm$ 0.02	1.15
Lungs*	ND	0.11	0.15	0.02	ND	0.22	ND	0.50
Kidneys*	ND	0.08	0.13	0.01	ND	0.22	0.01	0.45
Heart*	ND	0.05	0.14	0.02	ND	0.14	ND	0.35
Spleen*	ND	0.03	ND	ND	ND	0.08	ND	0.11
Brain*	ND	ND	0.01	0.03	ND	0.06	ND	0.10
Group B								
Adipose(n=3)	ND	0.94 $\pm$ 0.20	0.86 $\pm$ 0.20	ND	0.06 $\pm$ 0.02	2.31 $\pm$ 0.46	0.12 $\pm$ 0.02	4.29
Liver (n=3)	ND	0.19 $\pm$ 0.05	0.16 $\pm$ 0.05	0.03 $\pm$ 0.01	0.01 $\pm$ 0.00	0.33 $\pm$ 0.06	0.03 $\pm$ 0.01	0.75
Muscle(n=3)	ND	0.06 $\pm$ 0.01	0.14 $\pm$ 0.05	0.02 $\pm$ 0.01	0.01 $\pm$ 0.00	0.36 $\pm$ 0.36	0.02 $\pm$ 0.01	0.61
Lungs*	ND	0.04	0.07	ND	ND	0.11	ND	0.22
Kidneys*	ND	0.05	0.05	0.01	ND	0.10	ND	0.21
Heart*	ND	0.04	0.10	ND	ND	0.12	ND	0.26
Spleen*	ND	0.03	ND	ND	ND	0.03	ND	0.06
Brain*	ND	0.02	ND	ND	ND	0.02	ND	0.04
Group G								
Adipose(n=3)	ND	1.72 $\pm$ 0.20	2.44 $\pm$ 0.07	ND	0.13 $\pm$ 0.03	3.94 $\pm$ 0.05	0.19 $\pm$ 0.02	8.42
Liver (n=3)	ND	0.38 $\pm$ 0.10	0.48 $\pm$ 0.21	0.09 $\pm$ 0.02	0.01 $\pm$ 0.00	0.64 $\pm$ 0.20	0.04 $\pm$ 0.01	1.64
Muscle(n=3)	ND	0.16 $\pm$ 0.06	0.36 $\pm$ 0.05	0.05 $\pm$ 0.00	0.01 $\pm$ 0.00	0.54 $\pm$ 0.06	0.03 $\pm$ 0.01	1.14
Lungs*	ND	0.07	0.28	0.02	ND	0.28	0.02	0.66
Kidneys*	ND	0.08	0.18	0.03	0.01	0.22	0.01	0.53
Heart*	ND	0.02	0.09	0.02	ND	0.13	ND	0.26
Spleen*	ND	0.03	0.11	ND	ND	0.11	ND	0.25
Brain*	ND	0.02	0.06	0.01	ND	0.05	ND	0.14
Group H								
Adipose(n=3)	ND	1.75 $\pm$ 0.31	2.15 $\pm$ 0.88	ND	0.12 $\pm$ 0.02	3.43 $\pm$ 0.93	0.17 $\pm$ 0.04	7.62
Liver (n=3)	ND	0.33 $\pm$ 0.05	0.35 $\pm$ 0.07	0.05 $\pm$ 0.02	0.01 $\pm$ 0.00	0.54 $\pm$ 0.02	0.03 $\pm$ 0.00	1.32
Muscle(n=3)	ND	0.22 $\pm$ 0.09	0.38 $\pm$ 0.12	0.05 $\pm$ 0.03	0.01 $\pm$ 0.00	0.66 $\pm$ 0.32	0.03 $\pm$ 0.01	1.35
Lungs*	ND	0.03	0.19	0.01	ND	0.22	0.01	0.48
Kidneys*	ND	0.11	0.17	0.01	0.01	0.25	0.01	0.56
Heart*	ND	0.04	0.06	ND	ND	0.12	ND	0.22
Spleen*	ND	0.02	0.11	ND	ND	0.07	ND	0.20
Brain*	ND	0.03	0.05	0.02	0.01	0.04	ND	0.15

ND: Below the detection limit (< 0.005 ppm) in each tissue or organ.

¹ heptachlor, ² heptachlorepoxyde, ³ oxychlordane, ⁴ *trans*-chlordane, ⁵ *cis*-chlordane, ⁶ *trans*-nonachlor, ⁷ *cis*-nonachlor, ⁸ mean of 7 compounds

*: The samples from 3 mice were mixed together because of the small amounts.

No chlordanes were detected in the mice before exposure (2 groups) or in the mice maintained without exposure for 6 months (2 groups).

for 6 months (2 groups). Chlordanes were present at levels of 6.44-13.00 ppm (total of 2 tissues / 6 organs) in the exposed mice. The adipose tissue of 2 tissues / 6 organs contained the highest chlordanes. In the Group A and Group B, the ratio of the adipose tissue chlordanes to the liver chlordanes were approximately 5.6 and 5.6 times, followed by the muscle (1.1 and 0.8), lungs (0.5 and 0.3), kidneys (0.5 and 0.3), heart (0.4 and 0.3), spleen (0.1 and 0.1) and brain (0.1 and 0.1), respectively. In Group G and Group H, the ratio of the adipose tissue chlordanes to the liver chlordanes were approximately 5.4 and 5.8 times, followed by the muscle (0.8 and 1.0), lungs (0.4 and 0.4), kidneys (0.3 and 0.5), heart (0.2 and 0.2), spleen (0.2 and 0.2) and brain (0.1 and 0.1), respectively. The other groups also showed the same tendency.

These results showed that (a) there was no difference in the proportion of chlordane accumulation between the 1-6 month exposure periods, and (b) about 65 % of the chlordanes accumulated in the adipose tissue of the mice, a very high proportion compared to its levels in the other tissues and organs. In the liver and muscle, its proportion was 11 % and 10 % on average, respectively.

trans-Nonachlor, which accounted for only 14 % of the Chlordane exposure levels, showed a high proportion (Group A and Group B, 52 and 52 %; Group G and Group H, 45 and 45 %) of the chlordanes accumulated in the mice. The relative abundance of *trans*-nonachlor in the tissues and organs of the mice thus differed markedly from that in the air. Oxychlordane and heptachlorepoide, which are metabolites of Chlordane, accounted for 23 and 20 % (Group A), 21 and 21 % (Group B), 31 and 19 % (Group G), 29 and 21 % (Group H) of the accumulation in the mice, respectively. *trans*-Chlordane, which was present at a high level (38 %) in the air, accounted for only 2 % of the accumulation in the mice, and the proportion of *cis*-chlordane (28 % in the air) was only 1 %. Heptachlor (15 % in the air) was not detected in the mice, while the proportion of *cis*-nonachlor in the mice was 2 %, equivalent to its proportion in the air.

These results showed that *trans*-nonachlor, oxychlordane and heptachlorepoide accumulated in the tissues and organs of the mice at high levels when the mice were exposed to Chlordane at low levels. The other groups also showed the same tendency.

The dose-accumulation relationship is shown in Fig. 1. A dose-accumulation relationship indicating a significant positive correlation was observed between the exposure amount of Chlordane and the level of chlordanes in each tissue and organ except for the heart. The coefficients of correlation in the brain (0.9338), adipose (0.9079), liver (0.8406), lungs (0.8317), muscle (0.8207), kidneys (0.8204) and spleen (0.7130) were calculated and shown. The slope of the regression line in the adipose (0.00878), liver (0.00157), muscle (0.00137), lungs (0.00051), kidneys (0.00049), spleen (0.00019) and brain (0.00016) were derived, and the order agreed well with the order of chlordanes accumulation by each tissue and organ (as shown in Table 3) except for the heart.

These results showed that (a) the tendency of chlordanes accumulation was adipose $\gg$ liver $\geq$ muscle, and was observed to have a close dose-accumulation relationship; (b) the brain had a close dose-accumulation relationship but a slow accumulation of chlordanes; (c) the heart had no dose-accumulation relationship.

Discussion

Our previous study⁵⁾ found a maximum level of indoor air Chlordane in residential living rooms of $3.77 \mu\text{g}/\text{m}^3$. In surveys¹²⁻¹⁴⁾ performed in the United States, most of the Chlordane levels of indoor air were below $10 \mu\text{g}/\text{m}^3$. Therefore, the Chlordane level used in the exposure test in the present study (4.22 - $11.36 \mu\text{g}/\text{m}^3$) was similar to that of indoor air contaminated with Chlordane. Further more, although the main exposure route in the present study was the respiratory exposure, exposure routes from drinking, feed and grooming are unlikely because of breeding in exposure chamber.

The body weights of the mice exposed to chlordane at these levels over a period of 1-6 months were not different from those of the non-exposed mice. However, 6.44-13.00 ppm of chlordanes (total of 7 compounds) were detected in the exposed mice (2 tissues / 6 organs).

We found that the adipose tissue of 2 tissues / 6 organs contained the highest chlordanes, the ratio of the adipose tissue chlordanes to the liver chlordanes was approximately 5.6 times, followed by the muscle (0.9), lungs (0.4), kidneys (0.4), heart (0.3), spleen (0.2) and brain (0.1).

The main compounds of the accumulated chlordanes were *trans*-nonachlor (approximately 50 %), oxychlordane (approximately 25 %) and heptachlorepoide (approximately 20 %), which together accounted for nearly 100% of the accumulated chlordanes. In contrast, *trans*-chlordane, *cis*-chlordane and heptachlor, which are constituents of technical chlordane and present at high levels in the air, were scarcely present in the adipose tissue.

The chlordanes accumulation after a prolonged inhalation of indoor air contaminated by a low level of Chlordane have not been studied previously. Noguchi¹⁵⁾ found that in rats exposed to $2.84 \mu\text{g}/\text{m}^3$ Chlordane in the air for 3.5 hours, chlordanes were the highest levels in the adipose tissue; the second highest levels were in the liver, kidneys, lungs, muscle and heart, and low levels were shown in the spleen and brain. *trans*-Nonachlor and *cis*-nonachlor were excreted extremely slowly, and oxychlordane and heptachlorepoide were significantly accumulated in these rats. Khasawinah¹⁶⁾ reported that in monkeys and rats exposed to 100-1000 $\mu\text{g}/\text{m}^3$ Chlordane in the air for 90 days, the adipose tissue contained the highest chlordanes residues, and the ratio of the total adipose residues to the liver residues was 5-20. In the monkeys, *trans*-nonachlor was the dominant residue, comprising nearly 50 % of the total residue, whereas in the rats, oxychlordane metabolite was the major residue. In a study of rats orally administered 10 $\mu\text{g}/\text{kg}/\text{day}$ of chlordane using a stomach probe for 1 and 2 weeks, Takeda et al.¹⁷⁾ found that oxychlordane, *trans*-nonachlor and heptachlorepoide 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.¹⁸⁾ showed that when mice were orally administered a single dose of a mixture of *trans*-chlordane and *cis*-chlordane, these compounds rapidly disappeared from the tissues, whereas oxychlordane remained in several tissues even at 52 weeks after administration. The study of chlordane metabolism in animals by Tashiro and Matsumura¹⁹⁾ revealed that *trans*-chlordane is mainly metabolized via oxychlordane, and that *cis*-chlordane is metabolized via oxychlordane and by a direct hydroxylation reaction, indicating that oxychlordane is the main metabolite of

Figure 1 Dose-accumulation relationship between long-term exposure to Chlordane at indoor air level and tissue concentrations of chlordanes in the mice.

The abscissa shows the exposure amount of Chlordane, dose (the total level of the 5 compounds in the air $\times$ exposure period, Cf. Table 1), and the ordinate is the level of chlordanes in each tissue / organ (the total level of the 7 compounds, Cf. Table 3).

The results for the 2 non-exposure groups (dose = 0, chlordanes level in each tissue / organ = 0) are included in the graphs.

Chlordane. Heptachlor is mainly metabolized via heptachlorepoxyde²⁰. 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 mouse tissues and organs in the present study, while oxychlordane, the major metabolite of *trans*-chlordane and *cis*-chlordane, and heptachlorepoxyde, the major metabolite of heptachlor, were detected at high levels. In contrast, *trans*-nonachlor, which accounts 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 is metabolized in the metabolic pathway described above²⁰. However, our present findings suggest that *trans*-nonachlor is not readily metabolized.

In the present long-term exposure tests, chlordanes were accumulated in the mice and a dose-accumulation relationship was observed. The regression line is generally expressed as $y = ax + b$; where y is the accumulation level of chlordanes in each tissue / organ, x is the exposure level of Chlordane $\times$ exposure period,

and a and b are constant. The Chlordane level used in the exposure test in this study (4.22 – $11.36 \mu\text{g}/\text{m}^3$) did not vary by much.

Therefore, we conclude that the chlordanes (*trans*-nonachlor > oxychlordane > heptachlorepoxyde) accumulation in mice (adipose $\gg$ liver $\geq$ muscle) depends on the exposure period when exposed to Chlordane at the levels of contaminated indoor air.

We previously showed that indoor air in houses is contaminated by the vaporization of residual Chlordane⁹, which persists for a long time⁹. Therefore, in residential environments treated with Chlordane, the exposure to residents by vaporization of residual Chlordane may be long term. The results of the present study of mice indicate that even with a low level of Chlordane in indoor air, chlordanes may be steadily accumulated in the bodies of human residents with prolonged exposure. These findings suggest that it is necessary to investigate the risks presented by a prolonged exposure to organochlorine chemicals in residential indoor air.

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