

## **Levels of Chlordane, Oxychlordane, and Nonachlor in Human Adipose Tissues**

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Chlordane was used as a termiticide for more than twenty years in Japan. Chlordane is stable in the environment such as sediment and its bioaccumulation in some species of bacteria, freshwater invertebrates, and marine fish is large (WHO 1984). Many researches were done to elucidate the levels of chlordane and/or its metabolite oxychlordane in human adipose tissues (Biros and Enos 1973, Frank et al. 1988, and Williams et al. 1988). A comprehensive review concerning chlordane was recently provided by USEPA (1988). On the other hand, Japan authorities banned the use of chlordane in september 1986.

In the last paper, we reported that both water and sediment of the rivers around Saga city were slightly contaminated with chlordane (Hirai and Tomokuni 1989). In the present study, we investigated the levels of chlordane, oxychlordane and nonachlor in human adipose tissues.

### **MATERIALS AND METHODS**

Adipose tissue samples were obtained from four females and twenty males of autopsy patients at Saga Medical School Hospital in 1989. The subjects were nine months to eighty nine years old. Both abdominal adipose tissue and other adipose tissues attached to some internal organs were collected from one male. Abdominal adipose tissue was collected from four females and nineteen males. Adipose tissue attached to axillary lymph node was collected from one male whose abdominal adipose tissue was too scarce to collect even one gram.

About one gram of the tissue was used for the analysis. The pretreatment of the adipose tissue for the determination of chlordane using GC/MS consists of

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extraction with acetone and n-hexane, partitioning with acetonitrile, extraction with n-hexane, silicagel column chromatography and concentration by evaporation. The procedure for the micro clean up and concentration developed by us (Hirai and Tomokuni 1987) was applied at the final step to concentrate the samples into 20-30  $\mu$ L in the capillary.

GC/MS was used to determine the total amount of trans- and cis-chlordane (chlordane,  $C_{10}H_6Cl_8$ ), oxychlordane ( $C_{10}H_4Cl_8O$ ), and the total amount of trans- and cis-nonachlor (nonachlor,  $C_{10}H_5Cl_9$ ). The apparatus used was a JEOL-DX300 double focusing GC/MS system coupled with a JEOL-DA5000 computer system (JEOL LTD, Tokyo, Japan). The MS system was operated in the mode of electron impact ionization and selected ion monitoring (SIM) of positive ions. Two fragment ions were monitored for each analyte, i.e., m/z 372.827 and 374.824 for chlordane, m/z 388.803 and 390.800 for oxychlordane, and m/z 406.788 and 408.785 for nonachlor. Other analytical conditions were shown in Table 1. The relative standard deviation of the determination was less than 10% when the chlordane in an abdominal adipose tissue was determined seven times. The limit of determination was 0.2 ng/g, 1.0 ng/g and 0.1 ng/g for chlordane, oxychlordane and nonachlor, respectively. Standard addition method was employed at the final step of the sample pretreatment to correct both the effect of matrix on the detection and the recovery of analytes.

Table 1 GC/MS condition

Column	2% Silicone OV-1 on Uniport HP(60-80 mesh) 2.6 mm i.d. x 2 m
Carrier gas	He 30 mL/min
Injection temperature	230 °C
Column temperature	230 °C
Separator temperature	230 °C
Inlet temperature	230 °C
Chamber temperature	230 °C
Ionization voltage	70 eV
Ionization current	300 $\mu$ A
Acceleration voltage	3 kV
Main slit	1000
Collector slit	50
Secondary multiplier voltage	2.5 kV

## RESULTS AND DISCUSSION

We first investigated the levels of chlordane in the adipose tissues attached to main internal organs and

abdominal adipose tissue obtained from one male. The results are shown in Table 2. The subject was male of fifty-eight years old. There were small differences in concentrations of chlordanes, oxychlordane and nonachlor among seven tissues. Each component showed the highest level in the abdominal adipose tissue and the lowest one in the adipose tissue attached to kidney.

Table 2 Levels of chlordanes, oxychlordane and nonachlor (ng/g tissue) in the abdominal adipose tissue and in adipose tissues attached to internal organs

Organ	Chlordane	Oxychlordane	Nonachlor	Total
Abdominal adipose tissue	4.5	10.9	35	50
Colon	4.3	10.5	31	46
Intestine	3.9	10.7	30	45
Heart	4.0	9.6	28	42
Lymph node	3.8	8.9	28	41
Pancreas	3.6	9.4	26	39
Kidney	3.5	8.3	26	38

We second investigated the levels of chlordanes, oxychlordane, and nonachlor in the adipose tissue of twenty-four subjects. Figure 1 shows the levels of chlordanes, oxychlordane and nonachlor in the abdominal adipose tissue of four females and nineteen males and those in the adipose tissue attached to axillary lymph node of one male. The levels of chlordanes, oxychlordane and nonachlor ranged from 4.5 to 49 ng/g, 11 to 75 ng/g, and 29 to 230 ng/g, respectively. The level of chlordanes was similar to that obtained from Ontario residents in 1983 to 1984 whose level ranged from 5 to 91 ng/g extractable fat (Frank et al. 1988). The levels of oxychlordane and nonachlor were also similar to those obtained from six Ontario municipalities, where the level of oxychlordane ranged from 8 to 237 ng/g adipose tissue and that of trans-nonachlor ranged from 14 to 467 ng/g adipose tissue (Williams et al. 1988). The median values of chlordanes, oxychlordane and nonachlor were 9.5, 23 and 110 ng/g, respectively. Coefficients of correlation between chlordanes and oxychlordane, chlordanes and nonachlor, and oxychlordane and nonachlor were 0.61, 0.75 and 0.83, respectively. Significant positive correlation was observed between every two components at 0.01 level. No significant correlation was observed between age and levels of the components. No significant difference was observed between the level of each component of males and that of females.

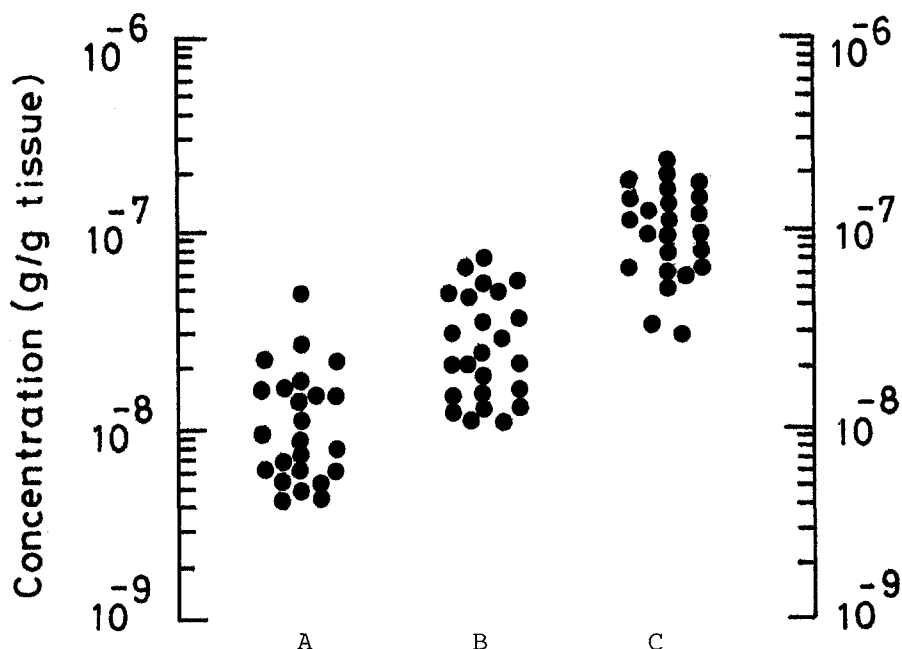


Figure 1 The levels of chlordanes(A), oxychlordanes(B) and nonachlor(C) in human adipose tissues

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