Bull. Environm. Contam. Toxicol. 25, 518-523 (1980)

# Chlordane Residues in Human Milk

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The occurrence of components of technical chlordane in environmental samples had received little attention until recently (ZITKO 1978). In the previous papers, MIYAZAKI et al. (1979, 1980) reported that cis- and trans-chlordane, cis- and trans-nonachlor, and oxychlordane had been identified in goby-fish (Acanthogobius flavimanus) collected from Tokyo Bay. Scope and limitation of the environmental pollution with chlordanes in Japan are of high interest. Chlordane residues were found in domestic animal tissues a well as aquatic fauna (YAMAGISHI). In the present study, samples of human milk have been collected in Tokyo area from 1978 to 1979, and also found to be contaminated.

## MATERIALS AND METHODS

Human milk samples were collected in several maternity hospital from women resident in Tokyo metropolitan area, from September 1978 to November 1979. All samples were immediately frozen until analysis. Analysis after extraction of fat was very similar to the method for fish and shellfish samples (MIYAZAKI et al.). A thawed sample (50g) was extracted with petroleum ether-ethyl ether, and the extract was cleaned up through acetonitrile-hexane partitioning and then applied to a Florisil column (25g, Floridin Co, 60-100 mesh, PR grade). After elution with 110 mL of hexane (discarded), the next hexane fraction (100 mL, Eluate A) gave trans-nonachlor, and subsequent 20% $CH_2Cl_2$ in hexane (200 mL, Eluate B) contained cis- and trans-chlordane, cis-nonachlor, and oxychlordane. Quantitation of chlordanes was carried out by gas chromatography using an electron capture detector (ECD-GC) under the following conditions. (1) column; 3 mm x 2 m glass column, packed with 1.5% OV-17 + 1.95% QF-1 on Chromosorb W (AW-DMCS) 80-100 mesh, temperature; injection and detector 250°C, column 200°C; carrier gas, $N_2$ 80 mL/min. (2) column; 3 mm x 2 m glass column, packed with 2% OV-210 on Shimalite W (AW-DMCS) 80-100 mesh, temperature; injection and detector 250°C, column 165°C; carrier gas, $N_2$ 75 mL/ min.

0007-4861/80/0025-0518 $01.20

(3) column; 3 mm x 3 m glass column, packed with 3% OV-1 on Gaschrom Q 80-100 mesh, temperature; injection 240°C, detector 270°C, column 180°C, carrier gas; $N_2$ 80 mL/min.

Recoveries from a fortified sample of cow's milk (50g) at 1 ppb each levels were in the range of 79% to 87% on this method.

The eluates A and B were pooled into annual fractions, respectively. The fractions were concentrated to 1 mL, and chlordanes were quantified by gas chromatograph-mass spectrometry (GC-MS) with multiple ion detection (MID). $M^+$ and $[M-Cl]^+$ peaks (and isotope peaks) of each chlordane residue were selected for mass monitoring. After purification of the fractions by thin layer chromatography on silica gel (Merck $F_{254}$, hexane), mass spectra were determined using a system, Hewlett-packard 5710A JEOL JMS-D 300 JMS 2000 System, on 3% OV-1, and 2% OV-210 as described previously (MIYAZAKI et al. 1980).

## RESULTS AND DISCUSSION

Oxychlordane and trans-nonachlor were present in all the samples of human milk collected from 1978 to 1979 in Tokyo metropolitan area. The levels ranged from 0.1 to 1 ppb (Table 1), and were much lower than those of p,p'-DDE and β-BHC (Fig.1). The chlordane residues in the pooled samples were confirmed by MID. Residues of cis- and trans-chlordane, and cis-nonachlor were also detected in the levels of 0.1 ppb. The peak patterns and retention times of those compounds by MID coincided with those of authentic specimen, and the concentrations were also aproximately equal to the values from ECD-GC. The peak closely before that of trans-nonachlor in the fragmentograms (Fig.2) on OV-210 column maybe correspond to the "third nonachlor isomer" found in human adipose tissue and also in technical chlordane (SOVOCOOL et al. 1975). This peak, however on OV-1 column, disappeared on the fragmentgrams of both samples.

After cleanup of the fractions (Eluates A and B) on silica gel plate, low resolution GC-MS analysis was carried out for further confirmation of these compounds. The mass spectra from human milk are shown in Figure 3, and essentially identical with those of authentic oxychlordane and trans-nonachlor, respectively.

Oxychlordane (SCHWEMMER et al. 1970, LAWRENCE et al. 1970, POLEN et al. 1971, STREET et al. 1972) is a mammalian metabolite formed from cis- and trans-chlordane, and trans-nonachlor (TASHIRO and MATSUMURA 1977, 1978),

Table 1. Chlordane Residues in Human Milk Samples in Tokyo (ppb on whole milk basis).

| Year | Number of sample | oxychlordane Range | Mean | MID | trans-nonachlor Range | Mean | MID | cis-nonachlor MID | trans-chlordane MID | cis-chlordane MID |
|---|---|---|---|---|---|---|---|---|---|---|
| 1978 | 11 | 0.1-1.0 | 0.48 | 0.42 | 0.1-0.9 | 0.54 | 0.47 | 0.06 | | |
| 1979 | 12 | 0.3-1.1 | 0.52 | 0.53 | 0.5-1.1 | 0.71 | 0.79 | 0.18 | 0.16 | 0.13 |



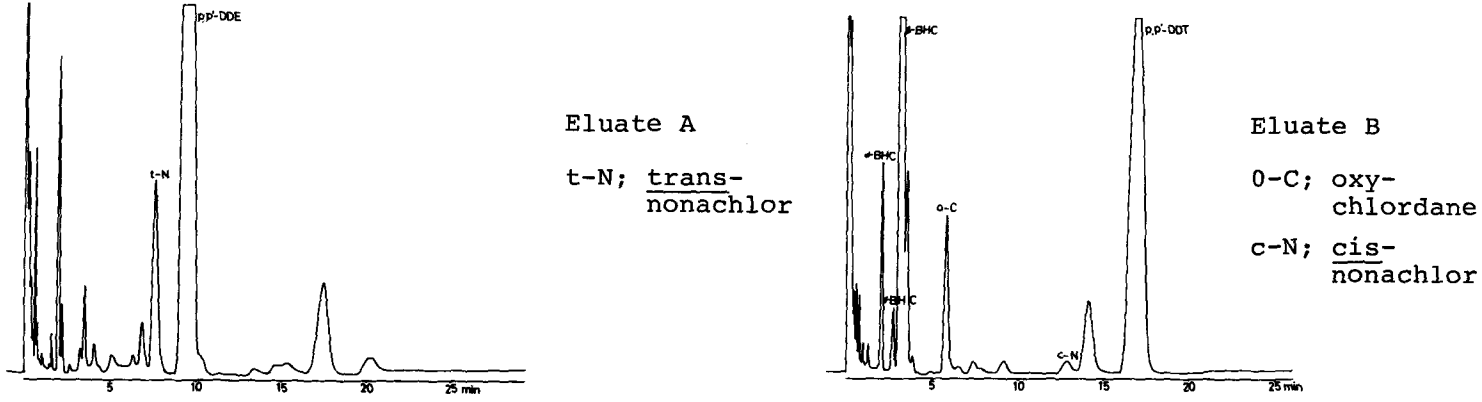



Figure 1. Typical ECD Gas Chromatograms of Eluates A and B from Human Milk (column; 1.5% OV-17 + 1.95% QF-1).

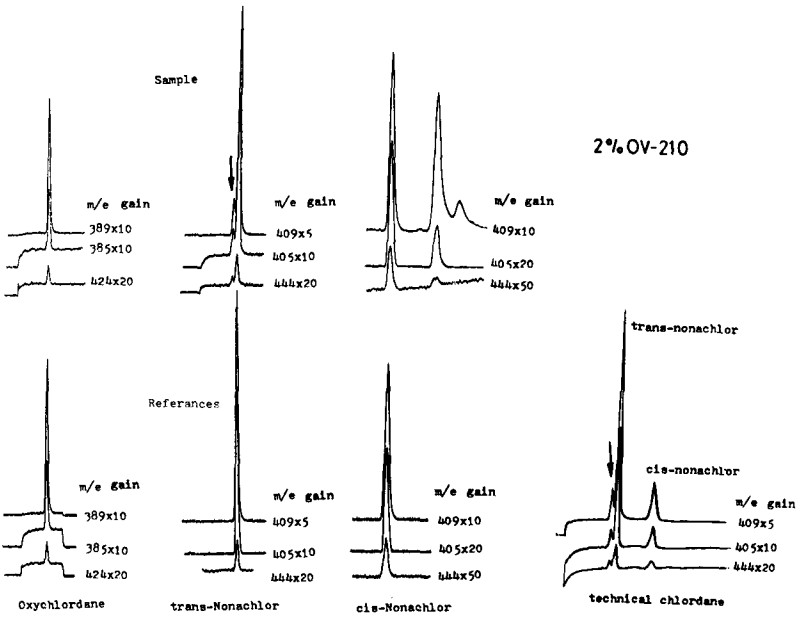


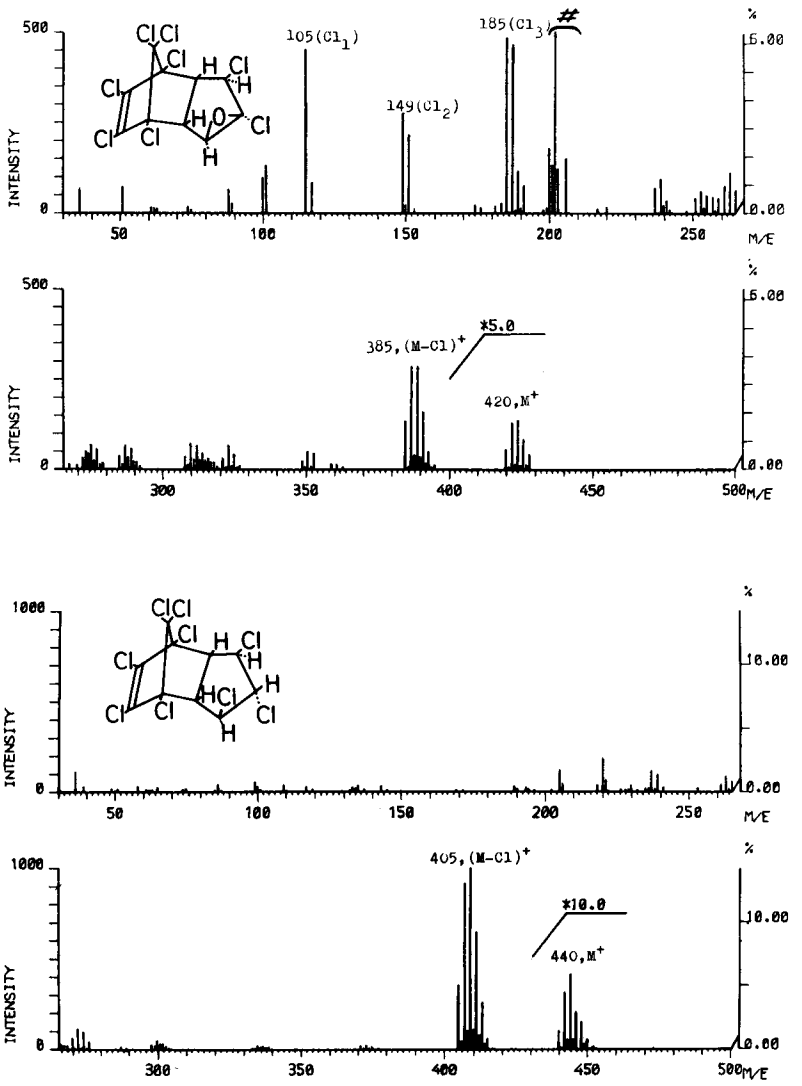

Figure 2. Mass Fragmentograms of Chlordane Residues in Human Milk.

(↓): Indicated peaks maybe correspond to the third nonachlor isomer.

and these compounds are the constituents of technical chlordane (SOVOCOOL et al. 1977) and technical heptachlor (COCHRANE et al. 1970). Therefore, the occurrence of the chlordanes in human milk is indicative of pollution with technical chlordane and/or heptachlor.

Chlordane (annual consumption, 500 tons) have been used for wood protection from termites and powder post beetles, however heptachlor was prohibited as pesticide use in 1973 in Japan. Consequently, the contamination of human milk with these compounds maybe attributed to technical chlordane.

Although KITAMURA et al. (1978) reported the detection of "dehydrochlordane" in adipose tissues of Japanese, we could not detect it in the milk samples. In U.S.A (STRASSMAN and KUTZ 1977) and Canada (MES and DAVIES 1979), the occurrence of oxychlordane and trans-nonachlor in human milk had been reported: U.S.A, oxychlordane 5 ppb, trans-nonachlor 1 ppb, 1973-1974; Canada, oxychlordane 1 ppb, trans-nonachlor 1 ppb,1975. The residue levels in human milk in Tokyo may be the same as or lower than those in U.S.A or Canada.




Figuere 3. Mass Spectra of Oxychlordane (upper) and trans-Nonachlor (lower) from Human Milk.

(#): Indicated peaks may be attributed to some co-extractives.

Oxychlordane levels in human milk were relatively higher than other chlordane residues, while high cis-chlordane and low oxychlordane levels had been observed in fish and shellfish from Tokyo Bay. trans-Nonachlor levels were also high in all of them. The component patterns of chlordane residues may reflect the differences in food chain and metabolic behavior, as shown in human and rat livers (TASHIRO and MATSUMURA 1978).

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