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# Residues of the Synergist S-421 in Human Milk Collected from the Tokyo Metropolitan Area

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S-421, 1,1'-Oxybis(2,3,3,3-tetrachloropropane), is a synergist for pyrethroid insecticides against sanitary insects such as mosquitos, houseflies and cockroaches (HAYASHI 1969). In a previous paper we reported on the identification of the synergist present in human milk samples collected in the Tokyo metropolitan area (MIYAZAKI et al. 1981), but not the method for the determination of S-421 residues. Therefore, we investigated the analytical method, and then examined the residual levels in human milk samples. As a result, a gas chromatographic method for residual organochlorine pesticides was found to be applicable to the synergist in human milk. The synergist was detected at relatively low levels in most human milk samples, and also found to be in use in some of commercial insecticide formulations. These results suggested that the presence of the synergist residue in human milk may be due to the use of these formulations containing the synergist.

## MATERIALS AND METHODS

### 1) Samples and Reagents

Human milk samples were collected at several maternity hospitals from women residing in the Tokyo metropolitan area during the period from September to November 1979 (12 samples) and 1980 (10 samples). All samples collected were immediately frozen until analysis. Reagents and solvents used were of pesticide grade or equivalent. S-421, reference compound, was as described previously (MIYAZAKI et al. 1981). Oxychlordane was purchased from Nanogen Co. Ltd. (U.S.A.), and trans-nonachlor was prepared by chlorination of heptachlor as described previously (MIYAZAKI et al. 1980). Other organochlorine pesticides were purchased from Wako Pure Chemical Industries Ltd. (Japan).

### 2) p-Values

p-Values of S-421 were determined by the method described by BOWMAN & BEROZA (1965).

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3) Analytical method for S-421 and organochlorine pesticides in human milk

A human milk sample which had been thawed (25g) was extracted with ethanol-ethyl ether-hexane (2:1:2, 50 mL x 3), and the extract was concentrated, dissolved with 30 mL of hexane, and partitioned with acetonitrile saturated with hexane (30 mL x 4). The combined acetonitrile extract was poured into 350 mL of 2% NaCl solution. The aqueous solution was extracted with 100 mL of hexane ( x 2). The hexane extract was washed with water ( 50 mL x 2), dehydrated with anhydrous $Na_2SO_4$ (10g), and concentrated to ca. 3 mL. The concentrate was chromatographed on a Florisil column (Florisil, 20g) with the following elution system: first fraction with 150 mL of hexane contained p,p'-DDE and trans-nonachlor; second fraction with 200 mL of 20% dichloromethane in hexane contained S-421, β-HCH, p,p'-DDT, and oxychlordane; third fraction with 200 mL of dichloromethane : acetonitrile : hexane (50.00 : 0.35 : 49.65) mixture contained dieldrin. Each fraction was concentrated to 5 mL and analyzed with a gas chromatograph equipped with an electron capture detector (ECD/GLC). The presence of S-421 was confirmed by multiple ion detection mass spectrometry (MID). The recovery of these organochlorine pesticides was by more than 80%. Reagent blanks showed no detectable pesticide or chlorinated organic residues.

4) Apparatus and operating conditions

GC analysis was carried out by using an ECD ($^{63}Ni$), and the operating parameters were as described in Table 1. GC/MS analysis was performed on a JEOL D-300 mass spectrometer equipped with a Hewlett-Packard 5710A gas chromatograph and a JEOL 2000 disc system under the following conditions: column, OV-1. 3% Chromosorb W (AW-DMCS), 80-100 mesh, 1.8 m x 2 mm(i.d.); column temperature 200°C, injection and separator temperature 250°C; EI 20 eV, He 25 mL/min.

5) Analytical method for S-421 in commercial insecticides formulations

Mosquito coils and mats (for an electric vaporizer) was pulverized and extracted with hexane. Aerosoles were sprayed on the filter paper and the paper was extracted with hexane. Each hexane extract was examined by ECD/GLC, and the detected samples were confirmed by GC/MS.

## RESULTS AND DISCUSSIONS

p-Values of S-421 were determined to be 0.19 for hexane/acetonitrile, and 0.07 for hexane/dimethyl formamide. These data indicated that hexane-acetonitrile partitioning, which had been conventionally used in the

residual analysis of pesticides, was applicable to determination of the residual synergist. The elution pattern of S-421 with column chromatography on Florisil was examined for a modification of the elution system described by MILLS et al.(1972). Consequently, after elution of polychlorinated biphenyl with 150 mL of hexane, the synergist was practically recovered in the second fraction with 200 mL of 20% dichloromethane in hexane. S-421 could be detected with a higher sensitivity by an ECD (minimum detectable 2 pg) because of eight chlorine atoms included in the molecule. The separation of S-421 from other contaminants in ECD/GLC is of importance. As shown in Table 1, the peak of the synergist could be separated from that of HCH isomers on OV-1 column. However, it is musked on OV-17 + QF-1 column. It is should be noted that the peak of the synergist overlapped with that of dibutyl phthalate on OV-1 column. Each column can be applied to the determination by MID because of differrence of its mass number.

Table 1. Relative Retention Times (aldrin=1.00) of S-421 and Contaminants

| Liquid phase | 3%OV-1 | 2%OV-17 | 1.5%OV-17 +1.95%QF-1 | 5%OV-210 |
|---|---|---|---|---|
| Support | *1 | *2 | *2 | *2 |
| Mesh | 80/100 | 80/100 | 80/100 | 80/100 |
| Column temp. | 180°C | 190°C | 200°C | 170°C |
| Inlet temp. | 270°C | 280°C | 270°C | 240°C |
| L x i.d.(cm) | 300x0.3 | 200x0.3 | 200x0.3 | 200x0.3 |
| $N_2$ (mL/min) | 80 | 90 | 70 | 80 |
| S-421 | 0.81 | 0.84 | 0.85 | 1.00 |
| α-HCH | 0.44 | 0.50 | 0.54 | 0.65 |
| β-HCH | 0.47 | 0.74 | 0.76 | 0.95 |
| γ-HCH | 0.51 | 0.64 | 0.68 | 0.82 |
| δ-HCH | 0.53 | 0.88 | 0.89 | 1.06 |
| DBP*3 | 0.84 | 1.05 | 1.09 | 2.23 |
| aldrin | 1.00 | 1.00 | 1.00 | 1.00 |
| (min) | (7.20) | (5.14) | (5.10) | (6.41) |

*1 = Gas Chrom Q *2 = Chromosorb W(AW-DMCS)
*3 DBP = Dibutyl phthalate

The developed method is thus similar to the one for common organochlorine pesticides, and therefore enables simultaneous residue analyses. The recovery from cows' milk samples fortified at 5 ppb on whole milk basis of S-421 was 95.4% (n=3, s.d.=1.7). S-421 residues are examined for the total of 22 human milk samples in 1979 and 1980. Quantitative analyses were carried out by ECD/GLC, and further confirmed by monitoring at the peak of m/z 132 and 134 by MID. Table 2

Table 2. Residual Levels of S-421 in Human Milk Samples in Tokyo Metropolitan Area by MID (ppb)

| Year | No. | Whole milk basis range | mean | s.d. | detection | Fat milk basis mean | s.d. |
|---|---|---|---|---|---|---|---|
| 1979 | 12 | nd*-3.8 | 1.5 | ± 1.2 | 9/12 | 32 | ± 27 |
| 1980 | 10 | nd -0.9 | 0.5 | ± 0.5 | 6/10 | 14 | ± 8 |

*nd < 0.2 ppb (whole milk basis)

shows the residual levels measured by MID. The levels were approximately equal to those measured by ECD/GLC, and foun to be 32 and 14 ppb on the fat milk basis in 1979 and 1980 respectively. In the same samples determined for S-421 at 1980 (n=10), the residual levels of organochlorine pesticides on the fat milk basis were as follow: β-HCH 1.73 ppm (s.d. = ±1.34 ppm), p,p'-DDE 1.42 ppm (s.d. = ±1.18 ppm), dieldrin 0.038 ppm (s.d. = ±0.030 ppm), trans-nonachlor 0.018 ppm (s.d. = ±0.011 ppm), oxychlordane 0.015 ppm (s.d. = ±0.008 ppm). Residual levels of S-421 were intensively less than those of p,p'-DDE and β-HCH, and about equal to those of oxychlordane and trans-nonachlor.

S-421 has been reported to be available as a synergist for pyrethroid insectisides against sanitary insects (HAYASHI 1969). Therefore, we examined the presence of the synergist in commercial insecticides. As shown in Table 3, S-421 was detected in some of aerosoles and mosquito coil samples, but not in mats for mosquito. We presumed that the presence of S-421 residue in human milk may be due to use of these commercial insecticide formulations containing this synergist.

Table 3. Detection of S-421 in Commercial Insecticides for Sanitary Insects

| formulations | detection |
|---|---|
| aerosoles (for mosquito & housefly) | 3/8 |
| mosquito coils | 2/5 |
| mats (for mosquito) | 0/8 |

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