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Identification of the Synergist *Bis*(2,3,3,3-tetrachloropropyl)ether in Human Milk

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Since 1971, we have monitored residual organochlorine pesticides in human milk in Japan (YAMAGISHI et al. 1979). MIYAZAKI et al. (1980) reported chlordane residues in human milk. During the gas chromatographic analysis, an unknown peak was found on the chromatogram closely after that of β-HCH in the case of using a column packed with 1.5% OV-17 + 1.95% QF-1. The relative retention time of the peak to aldrin was 0.85 on this column. This compound was almost unchanged by treatment with conc. H_2SO_4, and much more sensitive to an electron capture detector (ECD) than to a flame ionization detector (FID). This paper describes the identification of the peak.

MATERIALS AND METHODS

Human milk samples (total 550 g) were collected from several maternity hospitals from the women resident in the Tokyo metropolitan area, from September to November 1978. The cleanup method of the sample was the same as that described in MIYAZAKI et al. (1980). Briefly, the sample was extracted with petroleum ether-ethyl ether, and the extract was cleaned up through acetonitrile-hexane partitioning and then applied to a Florisil column (25 g, Floridin Co., 60-100 mesh). After washing the column with hexane (210 mL), the eluate with 20% dichloromethane in hexane (200 mL) was concentrated, further cleaned up by thin layer chromatography on silica gel (Merck F_{254}, hexane), and submitted to GC/MS analysis.

GC/MS analysis was performed on a JEOL JMS D-300 mass spectrometer equipped with a Hewlett-Packard 5710A gas chromatograph and a JEOL JMA 2000 data system under the following conditions: column, OV-1, 3% on Chromosorb W(AW-DMCS), 80-100 mesh, 1.8 m x 2 mm (i.d.); column temp. 190°C; injection and separator temp. 250°C; He 30 mL/min ; EI 70eV; CI, CH_4 at ca. 1 Torr.; high resolution measurements, resolution 5000, perfluorokerosene was used as a reference standard. GC analysis was carried out using a ^{63}Ni ECD and a FID under the conditions as shown in Table 3.

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Bis(2,3,3,3-tetrachloropropyl)ether (S-421) was prepared according to the method described by BECKE & SPERBER (1959); oily liquid, bp 146-150°C/1 mmHg (lit. bp_1 144-155°C). The mass spectrum of this compound in EI mode did not show the molecular ion $(M)^+$, but, in CI mode, gave the very weak $(M+1)^+$ ion. High resolution measurement (CI) at the $(M+1)^+$ ion afforded elemental composition of $C_6H_7O^{35}Cl_8$ (observed mass, m/e 374.8019, error 1.4 mmu), and led to $C_6H_6OCl_8$ as the molecular ion. In Table 1, the data of high resolution measurements (EI) at prominent ions are shown.

Table 1. High Resolution EI Measurements of Authentic S-421

Observed mass(m/e)	Error(mmu)	Assignment
78.9966	1.5	$C_2H_4O^{35}Cl_1$
82.9446	-0.7	$C_1H_1{}^{35}Cl_2$
84.9423	-0.1	$C_1H_1{}^{35}Cl_1{}^{37}Cl_1$
108.9616	0.7	$C_3H_3{}^{35}Cl_2$
110.9581	0.0	$C_3H_3{}^{35}Cl_1{}^{37}Cl_1$
129.9136	-0.6	$C_2H_1{}^{35}Cl_3$
131.9116	0.1	$C_2H_1{}^{35}Cl_2{}^{37}Cl_1$
133.9075	-0.7	$C_2H_1{}^{35}Cl_1{}^{37}Cl_2$

RESULTS AND DISCUSSION

Fig. 1 shows a typical ECD gas chromatogram of human milk samples. An unknown peak appears apart from the peak of β-HCH on 3% OV-1 column, whereas between the peaks of β- and δ-HCH on 2% OV-17 column. Fig. 2 shows the mass spectrum of the unknown compound. The fragmentation pattern indicates it to be an aliphatic compound containing a number of chlorine atoms. The isotopic distribution of the base peak at m/e 79 confirms the presence of one chlorine atom in it. High resolution

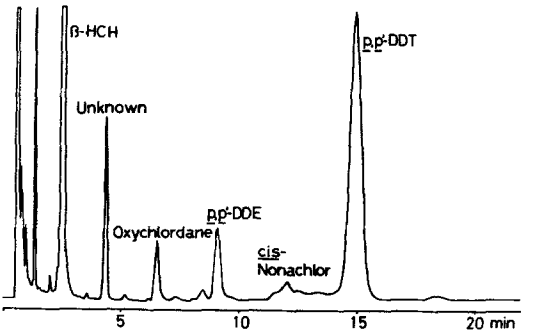

Fig. 1. Typical ECD Gas Chromatogram from Human Milk (3% OV-1)

Table 2. High Resolution EI Measurements of Unknown Peak

Observed mass(m/e)	Error(mmu)	Assignment
78.9962	1.1	$C_2H_4O^{35}Cl_1$
82.9455	0.0	$C_1H_1{}^{35}Cl_2$
108.9616	1.0	$C_3H_3{}^{35}Cl_2$
110.9568	-1.2	$C_3H_3{}^{35}Cl_1{}^{37}Cl_1$
129.9145	0.1	$C_2H_1{}^{35}Cl_3$
131.9105	-0.8	$C_2H_1{}^{35}Cl_2{}^{37}Cl_1$
133.9064	-1.8	$C_2H_1{}^{35}Cl_1{}^{37}Cl_2$

measurement of this base peak afforded elemental composition of $C_2H_4OCl_1$ (Table 2). Therefore, this compound was assumed to be a polychlorinated aliphatic ether on the basis of these spectral data. Especially, Bis(2,3,3,3-tetrachloropropyl)ether (S-421) was suspected as the aliphatic ether from the other informations. Thus, the unknown compound was identified as S-421 by comparison of its mass spectrum and retention times with those of an authentic sample (Fig. 2, Table 3). The data of high resolution measurements of the compound were in good agreement with those of S-421 (Table 1,2). Also, S-421 has been used as a synergist for pyrethrin insecticides at present.

The residual level of S-421 in pooled human milk was approximately 2 ppb on whole milk basis. The residues of S-421 in human milk may indicate previous exposure to insecticides including the synergist of S-421 from many possible sources. BECKE & SPERBER (1953) first prepared S-421, and it was reported as a synergist for insecticides (ADOLPHI 1958, HAYASHI 1969). Acute toxicity of S-421 is very low to rat (LD_{50} 3.6 g/kg) and rabbit (LD_{50} 2.5 g/kg)(NIOSH 1977), and its metabolites

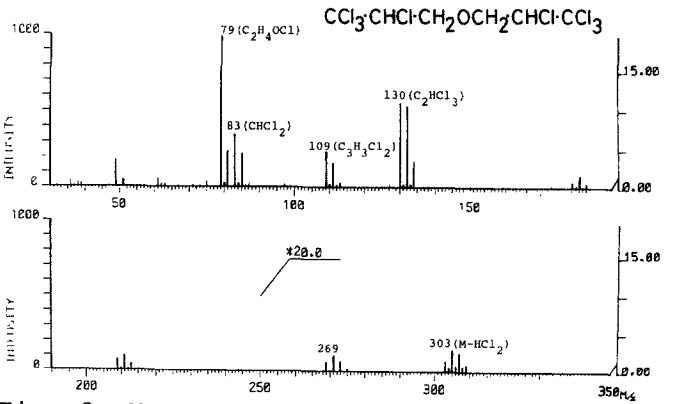

Fig. 2. Mass Spectrum of Unknown Compound Obtained from Human Milk (S-421)

Table 3. Retention Times of Unknown Peak, and S-421 (relative to aldrin)

Liquid Phase	3% OV-1	2% OV-17	1.5% OV-17 + 1.95% QF-1
Support	Gas Chrom Q	Chromosorb W(AW-DMCS)	Chromosorb W(AW-DMCS)
Inj. & Det. Temp.	270°C	280°C	270°C
Column Temp.	180°C	190°C	200°C
N_2 Flow (mL/min)	80	90	70
Unknown Peak	0.82	0.84	0.85
S-421	0.82	0.84	0.85
Aldrin	1.00	1.00	1.00
(min)	(7.20)	(5.14)	(5.10)

in rat are hydroxy derivatives, chlorinated hydrocarbons, ethers, acids, and cyclic derivatives (FISHBEIN et al. 1968). However, it is not known at present whether the residual level in human milk on this study would cause any functional damage to the internal organs.

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