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Identification of γ-Chlordene in Freshwater Fish from the Tama River (Japan)

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As part of an environmental monitoring program directed toward Tokyo Bay , we reported that the herbicide CNP (YAMAGISHI *et al.* 1978), and chlordanes and related compounds (MIYAZAKI *et al.* 1979, 1980) had been identified in fish and shellfish collected from the bay. Moreover, during gaschromatographic analysis of freshwater fish (*Zacco playtypus*) collected from Tama River discharged into Tokyo Bay, an unknown peak was found before that of *trans*-nonachlor on OV-17 column in PCB fractions. This peak was observed at 0.95, 1.10 and 1.35 relative retention times to aldrin on OV-1, OV-17 and OV-17/OV-210 columns, respectively. This paper reports the identification of the peak.

MATERIALS AND METHOD

Freshwater fish (*Zacco playtypus*) was collected at Noborito, the lower reaches of Tama River, on July 22, 1979. The samples (whole body, 196 g) were homogenized with sodium sulfate (200 g) and extracted with n-hexane (1 L). After evaporation to 30 mL, the hexane layer was shaken with acetonitrile (4 x 40 mL). The acetonitrile layer was poured into 2% NaCl solution (1.4 L), then the aqueous solution was extracted with hexane (2 x 200 mL). The hexane was washed with water, dried (Na_2SO_4) and concentrated to 5 mL. The concentrate was chromatographed on a Florisil column (30 g) which topped with Na_2SO_4 (10 g) in hexane. The first hexane fraction (100 mL) was discarded. The next hexane fraction (100-200 mL) was evaporated to 1 mL. The concentrate was chromatographed repeatedly, and the fraction was evaporated to 0.1 mL for GC-MS analysis.

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All solvents and reagents used were pesticide grade or equivalent. Florisil ; PR grade, 60-100 mesh, was activated at 650°C for 18 h and stored in a desiccator. γ-Chlordene, the reference compound, was kindly provided by Velsicol Pacific Limited(Tokyo, Japan).

GC analysis was carried out using ECD (^{63}Ni) and FID ; columns; OV-1; 3% on Chromosorb W AW DMCS 100-120 mesh, 3 m, OV-17; 5% on Chromosorb HP 80-100 mesh, 2 m, OV-17/OV-210; 1.5%/1.95% on Uniport HP 100-120 mesh, 3 m; all columns were 3 mm in I.D.; N_2 50-70 mL /min; column temperature 200-220°C, injection and detector temperature 280°C.

GC-MS analysis was performed with JEOL JMS-D300 JMS 2000 Disc System; EI 70 eV; column OV-17; 5% on Chromosorb HP 80-100 mesh, at 220°C, 2 mm x 1.8 m, He 30 mL/min; injection and separator temperature; 250°C.

RESULTS AND DISCUSSION

An ECD gas chromatogram of the fish sample is given in Fig.1. Earlier peaks are due to PCBs and the highest peak is *trans*-nonachlor.

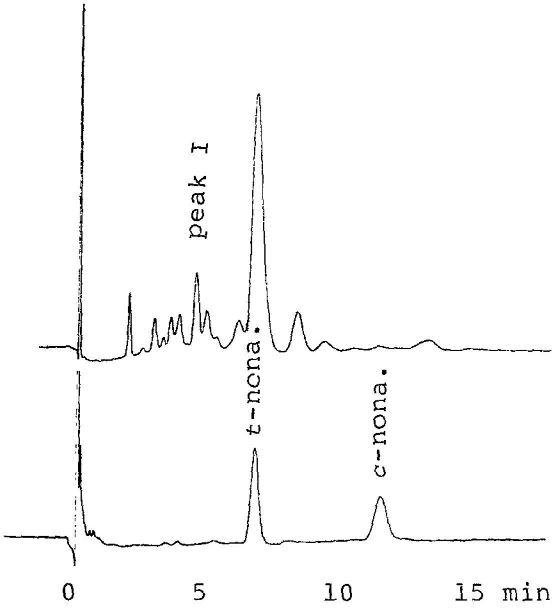

Fig. 1.
ECD-gas chromatograms of the PCB-fraction from freshwater fish and *trans* and *cis*-nonachlor.

OV-17 (5%)
210°C
N_2 50 mL/min

Preliminary tests to characterize substance was conducted. It was observed to be ; 1) much more sensitive to ECD than FID, 2) not effected by refluxing with methanolic KOH solution, 3) unchanged by conc. sulfulic acid treatment, and 4) extracted into acetonitrile on partitioning with hexane.

The fragmentation pattern is given in Fig.2. The characteristics of the fragmentation were chlorine clusters, m/e 230 (Cl_3, base peak), 265 (Cl_4), 301(Cl_5), and 336 (Cl_6, molecular peak), and not to exhibit retro-Diels-Alder peaks ; substituted cyclopentadiene fragments containing 0 to 6 chlorine atoms at m/e 66 to 270 (WILSON *et al.* 1977). Thus, the substance was identified as γ-chlordene (2,3,3a,4,5,8-hexachloro-3a,6,7,7a-tetrahydro-1,6-methano-1*H*-indene) by comparing with the spectrum of authentic specimen, and also in retention times (on OV-17, OV-1 and OV-17/OV-210 columns). β-Isomer was separated enough to distinguish on the OV-17 column.

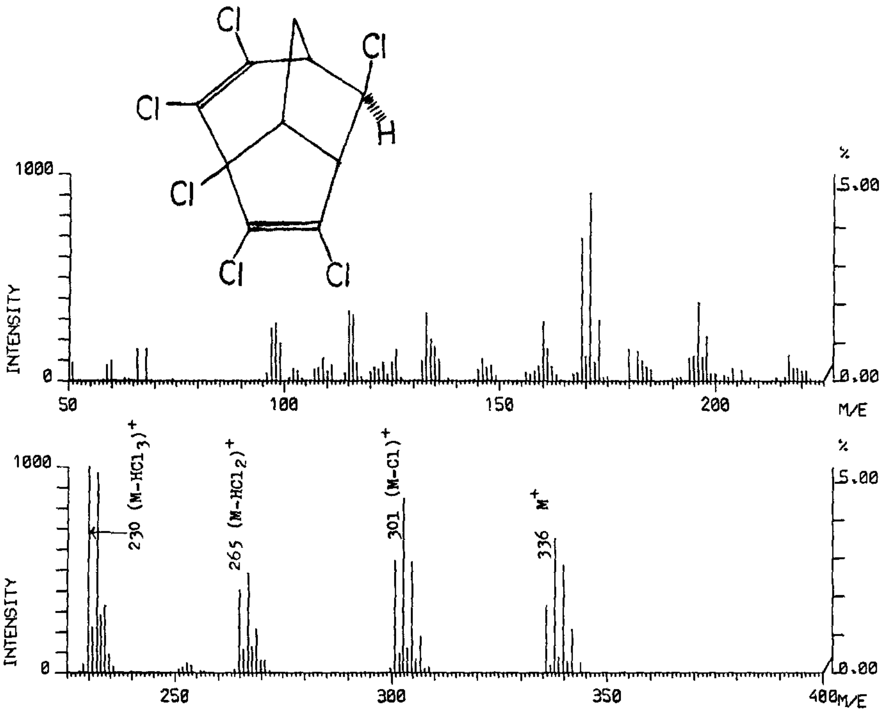

Fig. 2. EI mass spectrum of γ-chlordene in fresh-water fish.

γ-Chlordene has not previously been identified in environmental biota. This compound is one of the major constituents of technical chlordane (SOVOCOOL *et al.* 1977, PARLAR *et al.* 1979), may be formed by chlorine substitution while manufacturing of technical chlordane (GÄB *et al.* 1977).

In Japan, chlordane is used for controlling termite in wooden structures and products, and its consumption is estimated at 500 tons per year (HIROSE 197 7).

The approximate level of γ-chlordene in sample examined here was 23 ppb(wet basis). The levels of other organochlorine pesticides and PCBs in the sample were oxychlordane 21 ppb, *trans*-chlordane 39 ppb, *cis*-chlordane 91 ppb, *trans*-nonachlor 86 ppb, *cis*-nonachlor 24 ppb, heptachlor epoxide 24 ppb, total BHC (α,β-BHC) 225 ppb, total DDT(*p,p'*-DDE, *p,p'*-DDD) 66ppb, and PCBs 111 ppb. The levels of γ-chlordene seems relatively low compared with those of the other organochlorines. However, the data indicate that γ-chlordene is a common contaminant of aquatic fauna in this area.

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