Bull. Environm. Contam. Toxicol. 23,631-635 (1979)

Identification of *trans*-Nonachlor in Goby-Fish from Tokyo Bay

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As part of an environmental monitoring program directed toward Tokyo Bay, we reported that residues of the herbicide, CNP (1,3,5-trichloro-2-(4-nitrophenoxy)benzene) had been found in certain fish and shellfish (YAMAGISHI *et al.* 1978, 1979). We have also monitored pollution by other organochlorine pesticides and heavy metals reflected in fauna in this program (HORII *et al.* 1978). During gas chromatographic (GC) analysis of goby-fish samples, an unknown peak was found closely before that of *p,p'*-DDE on OV-1 column in PCB fractions. This peak was observed at 5.6, 5.8 or 5.0 min on OV-1, OV-17, or OV-210 columns, respectively. This paper reports the identification of the peak.

Preliminary tests to characterize the substance were conducted. It was observed to be: 1) much more sensitive to electron capture (ECD) than flame ionization detector (FID), 2) almost unchanged by conc. sulfuric acid treatment, 3) decomposed by refluxing with 1 *N* methanolic KOH solution, and 4) extracted into acetonitrile on partitioning with *n*-hexane.

MATERIALS AND METHODS

Goby-fish (*Acanthogobius flavimanus*) was collected at the seashore of Keihinjima along Tokyo Bay, on August 25, 1978. The samples (1.5 kg) were homogenized with sodium sulfate (1.5 kg) and extracted three times with *n*-hexane (1.5, 1.0, 0.5 L). After evaporation to 150 mL, the hexane layer was shaken with acetonitrile (3 x 240 mL). The acetonitrile was concentrated to 50 mL, poured into 2 % NaCl solution (500 mL), and in turn, the aqueous solution was extracted with hexane (2 x 200 mL). The hexane was washed with NaCl solution, dried (Na_2SO_4), and evaporated to 200 mL. The hexane was shaken with conc. sulfuric acid (3 x 80 mL), and water, dried, and concentrated to 5 mL. The concentrate was chromatographed on a Florisil column (2.3 x 10 cm) with *n*-hexane.

0007-4861/79/0023-0631 $01.00

The first hexane fraction (150 mL) was evaporated to 1 mL, further cleaned up by preparative thin-layer chromatography on polyamide (methanol), and submitted to GC-MS analysis.

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 dessicator. Polyamide: Merck$_{F254}$. *cis* and *trans*-Nonachlors, the reference compounds, were prepared as following; chlorine was bubbled through a refluxing solution of heptachlor (200 mg) in chloroform (35 mL) for 6 h, until no starting material was detected by FID-GC. After usual work up, the products were chromatographed on Florisil. Elution with *n*-hexane gave *trans* and *cis*-nonachlors successively. Recrystalized samples (hexane) gave no other peaks by GC; *trans*-nonachlor (32 mg), mp 128.2-128.5 (128-130°C, COCHRANE *et al.* 1970), *cis*-nonachlor (27 mg), mp 214.5 -215.2 (214-215°C, COCHRANE *et al.* 1970). Melting points were determined on a micro-hot stage and uncorrected.

GC analysis was carried out using a ^{63}Ni ECD and a dual FID; columns; OV-17; 2 % on Gas Chrom Q, 60-80 mesh, at 200°C, N_2 75 mL/min; OV-1; 2 % on Shimalite AW BW DMCS, 80-100 mesh, at 175°C, N_2 70 mL /min; OV-210; 2 % on Shimalite 80-100 mesh, at 170°C, N_2 60 mL/min; column sizes; glass columns, 3 mm x 2 m; injection and detector temperature; 240°C.

GC-MS analysis was performed with JEOL JMS-D300 JMS 2000 Disc System; EI; 70 eV; CI; CH_4 at 1 x 10^{-4} torr; column; OV-1; 3 % on Chromosorb W AW DMCS 100-120 mesh, at 210°C, 2 mm x 1.8 m, He 30 mL/min; injection and separator temperature; 250°C.

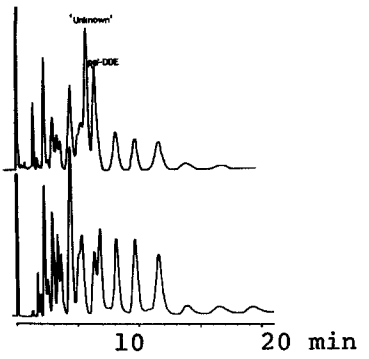

Fig. 1. ECD-gas chromatograms of the PCB-fraction from goby-fish and a mixture of KC-400 and KC-500 (1:1).

OV-1 (2 %)
175°C
N_2 70 mL/min

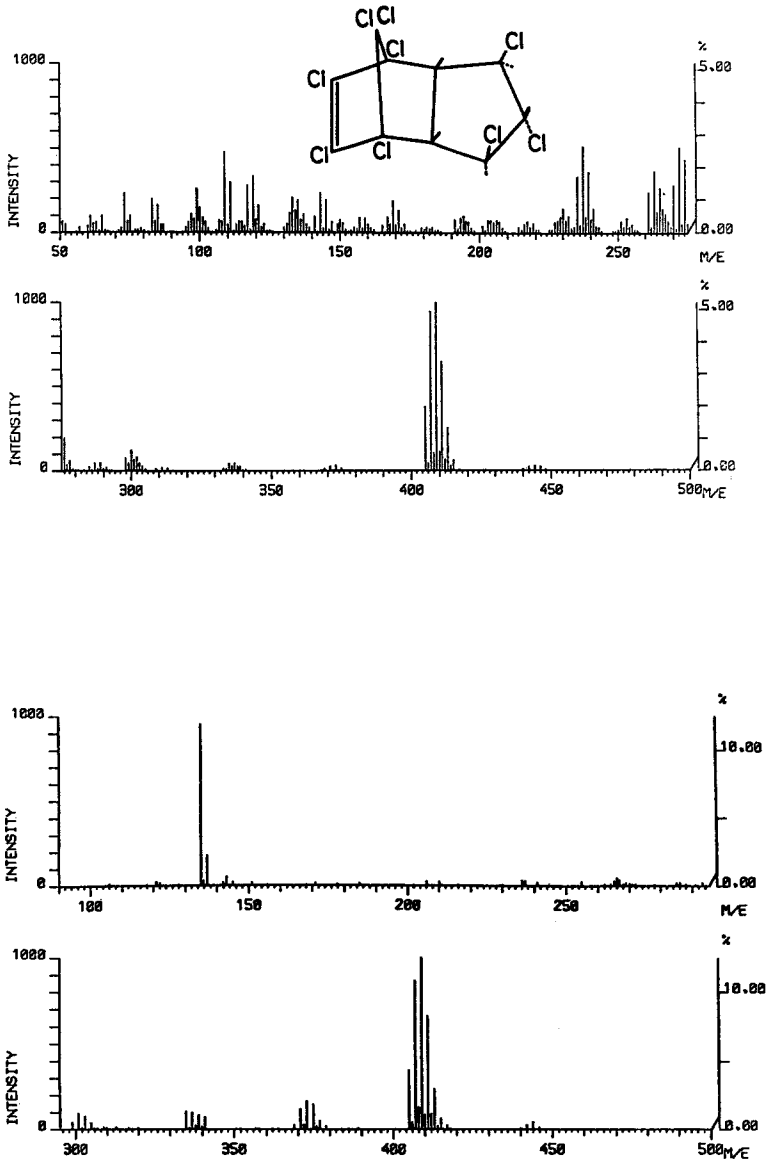

Fig. 2. EI (upper) and CI (lower) mass spectra of *trans*-nonachlor in goby-fish.

RESULTS AND DISCUSSION

The fragmentation pattern of this substance had the characteristics of Cl_8 (m/e 405, base peak 409), Cl_5 (m/e 270), and other chlorine-clusters of low intensities up to Cl_9 (m/e 440) as shown in Fig. 2. The high resolution measurements of the peak, m/e 405, led to a tentative formula, $C_{10}H_5Cl_8$ (M-Cl, observed: 404.7910, calculated: 404.7900), therefore $C_{10}H_5Cl_9$ as the molecular peak. Thus, the substance was identified as *trans*-nonachlor by comparing with the reference compounds in EI and CI mass spectra, and also in retention times (3.3 min for the substance and authentic *trans*-nonachlor, whereas 4.5 for the *cis*-isomer on OV-1 column). The mass spectra of *cis* and *trans*-isomers were essentially identical with that of the substance. The CI spectra also resembled the EI spectra. The clusters of m/e 135 ($C_5H_5Cl_2$) and 369 (Cl_7, M-Cl-HCl) were distinct, although the molecular peak remained weak as in the EI spectra, in accord with the literature (BIROS *et al.* 1972).

trans-Nonachlor has not previously been identified in environmental biota in Japan, while in U.S.A. widely detected in human adipose samples (KUTZ *et al.* 1976), and in some environmental samples (ZITKO AND SAUNDERS 1979, LAW AND GOERLITZ 1974, LICHTENSTEIN 1971). This compound is one of the major constituents of technical chlordane (SOVOCOOL *et al.* 1977), and technical heptachlor (COCHRANE *et al.* 1970), and the detection of *trans*-nonachlor may be indicative of pollution by these related compounds. In Japan, heptachlor was prohibited in 1973, and chlordane is used for wood protection from termite and powder post beetles, and the annual consumption is about 500 tons.

The approximate level of *trans*-nonachlor in gobyfish examined was 18 ppb and comparable with *p,p'*-DDE (29 ppb), while PCBs was higher (670 ppb). Because of relatively little capacity of human liver in metabolizing this compound (TASHIRO AND MATSUMURA 1978), and carcinogenicity of chlordane (EPSTEIN 1976, NATIONAL CANCER INSTITUTE 1977), it seems necessary to assess the environmental pollution by these compounds.

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