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Identification of Chlordanes and Related Compounds in Goby-fish from Tokyo Bay

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We recently reported that trans-nonachlor had been identified in goby-fish collected from Tokyo Bay (MIYAZAKI et al. 1979). The detection of this compound in fauna sugested environmental pollution by chlordane and/or heptachlor, because trans-nonachlor is one of the constituents of technical chlordane (SOVOCOOL et al. 1976) and technical heptachlor (COCHRANE et al. 1970). Then, the goby-fish samples elucidated to be contaminated with this compound have been intensively examined. Four unknown peaks were found in the fractions of organochlorine pesticides, while trans-nonachlor in the PCB fractions, using gas chromatography with electron capture detection (ECD-GC) (Fig. 1). The unknowns were identified as oxychlordane, trans and cis-chlordanes and cis-nonachlor, as were expected, by gas chromatography-mass spectrometry (GC-MS). These compounds have not been previously identified in environmental biota in Japan, while detected only in U.S.A.

EXPERIMENTAL

Goby-fish (Acanthogobius flavimanus) was collected at the seashore of Keihinjima along with Tokyo Bay, on August 25, 1978. The samples were the same as being treated in the previous work for the identification of trans-nonachlor (MIYAZAKI et al. 1979). In the present work, another fraction from Florisil chromatography was submitted to the examination; the samples (1.5 kg) were homogenized and extracted with n-hexane. After cleaning-up through partitioning between n-hexane and acetonitrile, treatment with conc. sulfuric acid, and Florisil chromatography (2.3 x 10 cm), trans-nonachlor and PCBs were eluted in the first n-hexane fraction, as described previously. Fig. 1 illustrates the ECD gas chromatogram from the next n-hexane-ether (4:1) fraction (200 mL). P-1, 2, 3, and 4 are the unknown peaks, and their retention times are shown in Tab. 1.

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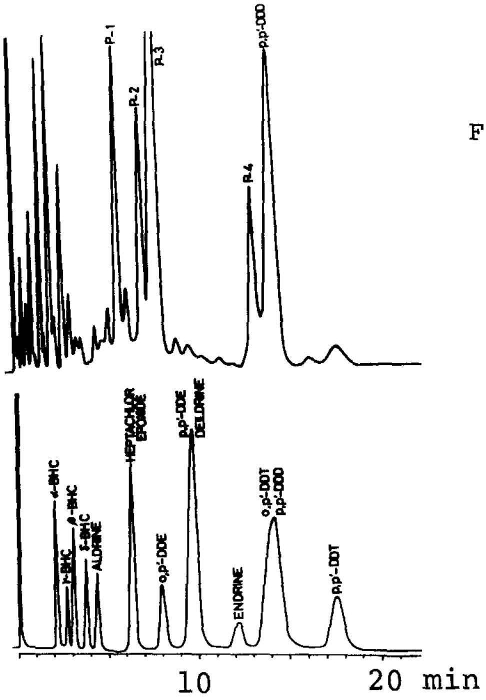

Fig. 1. ECD gas chromatogram of the organochlorine pesticide fraction from goby-fish and the reference compounds.

OV-17 (2 %)
200°C
N_2 75 mL/min

The concentrate was further cleaned-up by successive chromatography on polyamide (methanol) and silica gel plates (n-hexane). Three fractions were obtained by checking with GC; fraction A (P-4), B (P-2), and C (P-1 and 3). These fractions were submitted to GC-MS analysis.

Reagents and solvents used were of pesticide grade or equivalent. Polyamide and silica gel plates: Merck F_{254}. cis and trans-Chlordanes, and oxychlordane, the reference compounds, were also commercially available (Nanogen International Co. U.S.A.). cis and trans-Nonachlors were prepared by chlorination of heptachlor as described previously (MIYAZAKI et al. 1979).

GC analysis was carried out using a ^{63}Ni ECD and 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-D 300 JMS 2000 Disc System; EI 70 eV; CI; CH_4 at 1×10^{-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.

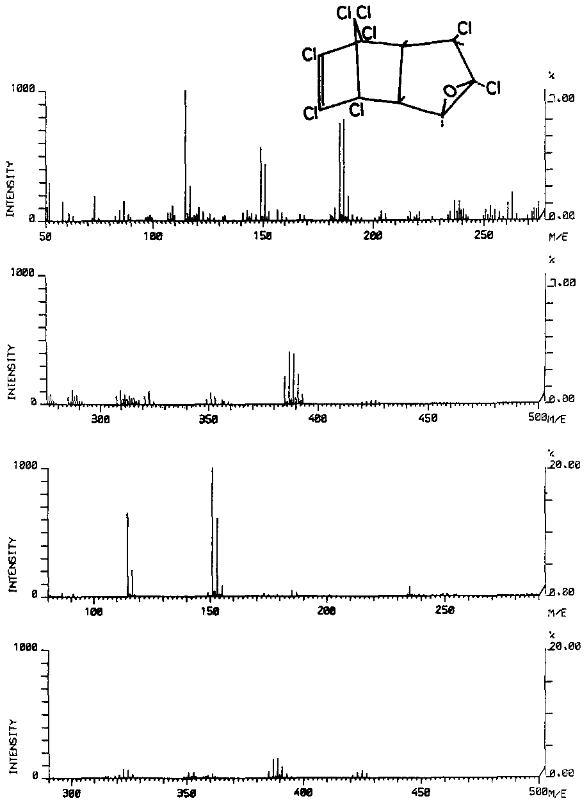

Fig. 2. EI (upper) & CI (lower) mass spectra of oxy-chlordane in goby-fish (P-1).

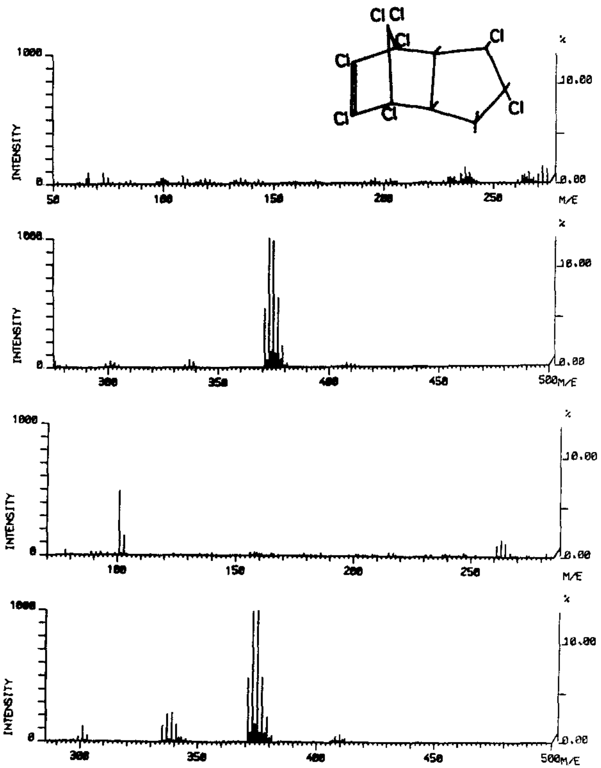

Fig. 3. EI (upper) & CI (lower) mass spectra of *trans*-chlordane in goby-fish (P-2).

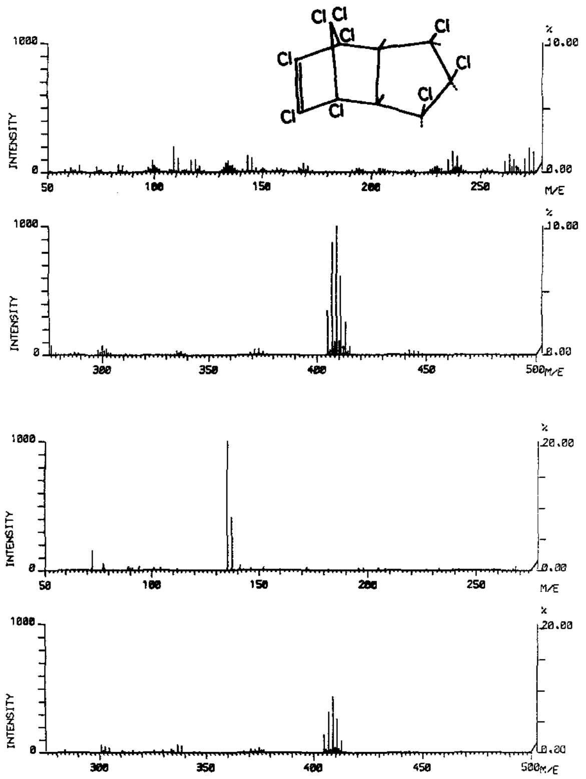

Fig. 4. EI (upper) & CI (lower) mass spectra of <u>cis</u>-nonachlor in goby-fish (P-3).

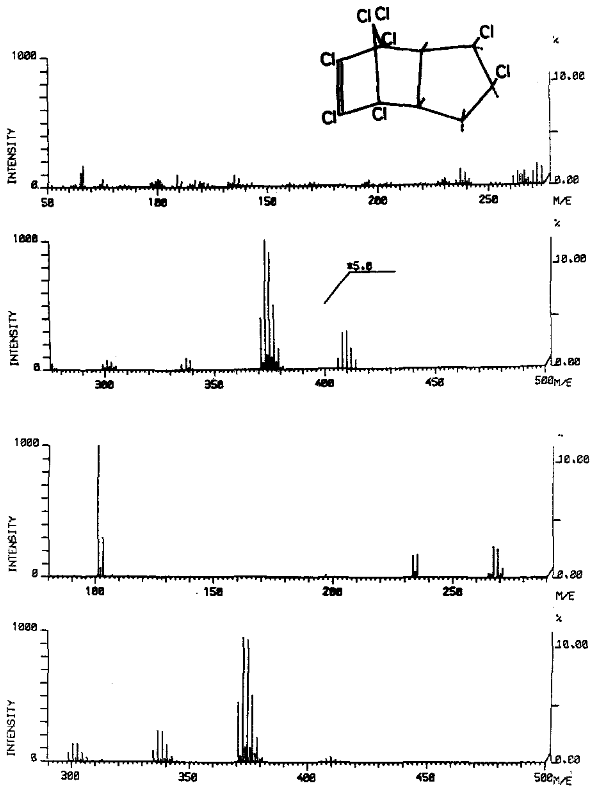

Fig. 5. EI (upper) & CI (lower) mass spectra of <u>cis</u>-chlordane in goby-fish (P-4).

RESULTS AND DISCUSSION

It was observed to be; all these unknowns were unchanged by treatment with conc. sulfuric acid, and except for P-2, decomposed on refluxing with 1 N methanolic KOH solution. The clean-up method adopted was based on these observations, and permitted GC-MS analysis without severe interferences.

TABLE 1

Retention times [min, and relative to aldrine (lower)] of the unknown peaks.

Peak	P-1	P-2	P-3	P-4
Assignment	Oxychlordane	*trans*-Chlordane	*cis*-Nonachlor	*cis*-Chlordane
OV-17	4.32 (1.32)	5.40 (1.66)	9.70 (2.98)	5.90 (1.81)
OV-1	4.31 (1.29)	4.88 (1.46)	8.58 (2.55)	5.47 (1.63)
OV-210	4.33 (1.59)	5.03 (1.85)	8.32 (3.04)	5.53 (2.03)
OV-1 (GC-MS)	2.67	2.92	4.45	3.17

The EI fragmentation pattern of P-1 had the characteristics of the chlorine-clusters, m/e 115 (Cl_1, base peak), 149 (Cl_2), 185 (Cl_3), 385 (Cl_7), and the weak peaks around 420 (Fig. 2). The high resolution measurement of the peak, m/e 385 led to a tentative formula, $C_{10}H_4OCl_7$ (M-Cl), and therefore $C_{10}H_4OCl_8$ as the molecular peak (Tab. 2). Thus, the substance, P-1 was identified as oxychlordane by comparing with authentic specimen in EI and CI mass spectra, and also in retention times (Tab. 1). The CI spectrum was similar with EI spectrum, but the peaks around m/e 185 (Cl_3) were very weak, and the intense peaks around 151 instead of 147 (Fig. 2).

GC-MS analysis of P-2 in EI mode gave the characteristic chlorine-clusters of m/e 371 (Cl_7, base peak), 355 (Cl_6), 299 (Cl_5), 270 (Cl_6), and very weak peaks around 406, and in CI mode, gave a similar spectrum, except for the intense peaks around m/e 101 (Cl_1) as shown in Fig. 3. The high resolution measurement of the peak m/e 371, afforded tentative formulae, $C_{10}H_6Cl_7$ (M-Cl) and $C_{10}H_6Cl_8$ as the molecular peak. From these data and comparison with those of the reference compounds, P-2 was identified as *trans*-chlordane (Tabs. 1 and 2).

TABLE 2

High resolution measurements of the unknown peaks

Unknown peak	Selected ion (m/e)	Observed (m/e)	Error (mmu)	Assignment
P-1	385	384.8114	-3.2	$C_{10}H_4OCl_7$ (M-Cl)
P-2	371	370.8257	-3.0	$C_{10}H_6Cl_7$ (M-Cl)
P-3	405	404.7939	4.0	$C_{10}H_5Cl_8$ (M-Cl)
P-4	371	370.8291	0.1	$C_{10}H_6Cl_7$ (M-Cl)

P-3 was identified as *cis*-nonachlor based on the following results; the fragmentation patterns of P-3 were identical with those of *cis* and *trans*-nonachlors in EI and CI modes; EI; chlorine-clusters of Cl_9 (m/e 440, M^+), Cl_8 (m/e 405, base peak 409, M-Cl) and Cl_5 (m/e 270); CI; in addition to them, Cl_2 (m/e 135) and Cl_7 (m/e 369); the retention time on OV-1 was identical with that of *cis*-nonachlor, but not of the *trans*-isomer (Tab. 1).

Mass spectra of P-4 were essentilly identical with those of *cis* and *trans*-chlordanes in EI and CI modes. The retention time on OV-1 column eliminated the trans-isomer, and the peak was identified as *cis*-chlordane (Tab. 1).

The weak molecular and the intense P-Cl peaks were common to these chlorine compounds in both EI and CI spectra. *retro*-Diels-Alder fragments of cyclopentene moieties were also observed in CI spectra; m/e 115 (C_5H_4OCl) from oxychlordane, 101 (C_5H_6Cl) from *cis* and *trans*-chlordanes, and 135 ($C_5H_5Cl_2$) from *cis* and *trans*-nonachlors, in accord with the literature (BIROS *et al*. 1972).

The residue levels of these organochlorine compounds in the samples were as following; oxychlordane 3, *trans*-chlordane 9, *cis*-chlordane 6, *cis*-nonachlor 8, *trans*-nonachlor 18, and heptachlor epoxide 1 ppb; the levels of total chlordane including oxychlordane were coparable with P,P'-DDE (29 ppb), while PCBs was higher (670 ppb).

In Japan, chlordane is used for wood protection from termite and powder post beetles (annual consumption, 500 tons) and heptachlor was prohibited in 1973. Occurence of chlordanes and these related compounds in environmental biota has not been previously demonstrated in Japan, while in U.S.A., detected in human adipose tissue (BIROS AND ENOS 1973, KUTZ *et al*. 1976, ATALLAH *et al*. 1977) and in some environmental samples (ZITKO AND SAUNDERS 1979, LAW AND GOERITZ 1974, LICHTENSTEIN 1971). Oxychlordane is a mammalian metabolite of *cis* and *trans*-chlordanes and *trans*-nonachlor (STREET AND BLAW 1972, BARNETT AND DOROUGH 1974, LAWRENCE *et al*. 1970). Human liver has relatively little capability in converting *trans*-nonachlor to *trans*-chlordane compared with rat liver TASHIRO AND MATSUMURA 1978), and the difference in the metabolic behavior of species may be reflected in the types and degrees of accumulation. Although, the mechanism, scope, and limitation of the pollution by these compounds are not still uncovered, goby-fish seems to be a sensitive indicator of environmental pollution. Goby-fish is a tolerant species to water pollution, easy to collection, and maybe capable of bioaccumulation related to its food-chain (nectobenthos, feed on sandworm).

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