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Gas Chromatographic Method for the Determination of Residues of Chlordanes and Related Compounds in Fish and Shellfish

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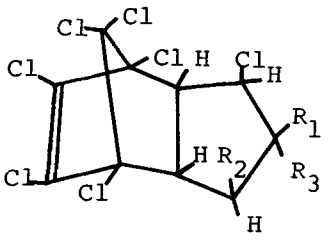
MIYAZAKI et al. (1970, 1980) found residues (chlordanes) of oxychlordane (o-C), cis- and trans-chlordane (c-C, t-C), and cis- and trans-nonachlor (c-N, t-N) in goby-fish collected from Tokyo Bay (Fig. 1). The cis- and trans-chlordane and nonachlor are the main constituents of technical chlordane (SOVOCOOL et al. 1977) and technical heptachlor (COCHRANE et al. 1970). o-C is a metabolite of c-C, t-C, and t-N (TASHIRO and MATSUMURA 1978). In Japan, heptachlor was prohibited in 1973, and 500 tons per year of technical chlordane has been used for wood protection from termites and powder post beetles. However, no data have been reported on residue levels of chlordanes in environmental biota in Japan.



	R_1	R_2	R_3
cis-nonachlor (c-N)	Cl	Cl	H
trans-nonachlor (t-N)	H	Cl	Cl
oxychlordane (o-C)	Cl	-O-	
cis-chlordane (c-C)	Cl	H	H
trans-chlordane (t-C)	H	H	Cl

Figure 1. Structure of Organochlorine Compounds

A few papers have described residue methodology for some of these compounds: U.S. Environmental Protection Agency (EPA) prescribed an electron capture gas chromatographic method (ECD-GC) for o-C, t-N and heptachlor epoxide in human adipose tissues (THOMPSON 1974). However, ECD-GC sometimes afforded poor quantifications of these compounds in environmental biota due to co-extractives such as PCBs and p,p'-DDE (ATALLAH et al. 1977, ZITKO 1978). Further cleanup or derivatization procedures (THOMPSON 1974, HOLDRINET 1979) before ECD-GC are not applicable to all of these compounds, nor always afford adequate selectivity, compared with selected ion

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monitoring mass spectrometry (SIM), which is less convenient (ZITKO 1978, SOVOCOOL et al. 1975).

This paper deals with an ECD-GC method for routine analysis of chlordanes in fish and shellfish in the presence of PCBs and common organochlorine pesticides. Florisil column chromatography of the sample extract gives a t-N fraction and the other chlordane fractions. The interference with organochlorines is also eliminated. These fractions are subjected to ECD-GC.

MATERIALS AND METHOD

1) Reagents

All solvents were pesticide grade. c-C, t-C and o-C were purchased from Nanogen Co. Ltd. (U.S.A.). c-N and t-N were prepared by chlorination of heptachlor as described previously (MIYAZAKI et al. 1979). Other organochlorine pesticides were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan); α-, β-, γ- and δ-BHC, p,p'-DDT, p,p'-DDE, p,p'-DDD, o,p'-DDT, o,p'-DDE, aldrin, endrin, dieldrin and heptachlor epoxide. Florisil, Floridin Co. (U.S.A), 60-100 mesh, PR grade, was activated at 650°C for 18 h and then stored in a desiccator. GC-column packings were supplied by Shinwakako Co. Ltd. Packings Center (Kyoto, Japan).

2) Apparatus and Operating Condition

Gas chromatograph, Shimadzu 5AP$_3$FE, equipped with a ^{63}Ni (10 mci) ECD; operating parameters are listed in Table 1.

GC-MS; Hewlett Packard 5710A, JEOL-D300, JMA-2000 system. Determination of t-N by SIM was carried out as follows: column; 3% OV-1 on 100-120 mesh Gas Chrom Q, 2 mm x 1.8 m; column temp.; 220°C; inj. and separator temp.; 250°C; He; 25 mL/min; ionization voltage; 70 eV; selected ion at m/e 408.8 (M-Cl).

3) Proposed Procedure

(Extraction of Sample)

Fish: Homogenized sample (20g) is dehydrated with anhydrous Na_2SO_4 (3-4 x weight of fish), and extracted with n-hexane (150, 50, 50 mL). The combined hexane is concentrated under reduced pressure in a Kuderna-Danish evaporator below 40°C. The concentration procedures described in this method are always carried out as this. The residue is dissolved in hexane (15 mL), and extracted with CH_3CN saturated with hexane (30 mL x 3). The acetonitrile is poured into 2% NaCl solution (350 mL), and the solution is shaken with hexane (100 mL x 2). The hexane is washed with 2% NaCl solution (50 mL), and dried over anhydrous Na_2SO_4, then concentrated to ca. 3 mL.

Shellfish: Homogenized sample (20g) is mixed and shaken with methanol (40 mL) and hexane (160 mL), and then allowed to stand overnight at a room temperature. This solution is centrifuged at 2500 rpm for 5 min, and filtered. The residue is rinsed with hexane (20 mL). The combined hexane is washed with water (30 mL x 2), dried over Na_2SO_4, and concentrated to ca. 3 mL.

(Florisil Column Chromatography)

A column (2.2 x 30 cm) is packed with Florisil (25g), and topped with Na_2SO_4 (8g) in hexane. The concentrate is chromatographed on Florisil. The first hexane fraction (110 mL) is discarded. The next hexane fraction (110-210 mL) contains t-N; Fr.A, and the third fraction with 20% CH_2Cl_2 in hexane (150 mL) contains o-C, t-C, c-C and c-N; Fr.B. Each fraction is concentrated to an appropriate volume.

(Gas Chromatography)

Each fraction is analyzed by ECD-GC: Fr.A on 2% OV-1, and Fr.B on 2% OV-17 or the mixed column, 1.5% OV-17 + 1.95% QF-1, as indicated in Table 1.

RESULTS AND DISCUSSION

Gas Chromatography

These chlordane derivatives could be separated on an OV-275 column, though overlapping with BHCs and p,p'-DDE. Retention times of t-N were considerably sensitive to the polarity of the liquid phase: on the nonpolar column, OV-1, the order was o-C, t-C, c-C, t-N and c-N with increasing retention times, and except t-N, this tendercy was retained even on OV-275, the most polar column among them, and the t-N peak shifted forward to the front of c-C peak on the OV-17 and more polar columns (Table 2). The chlordanes other than t-N could be separated on all the columns examined except the mixed column, DEGS + H_3PO_4, however high levels of PCBs interfered on them. Because of no interference from PCBs and common organochlorines after Florisil chromatography as Fig. 2 shows, OV-1 was selected for quantification of t-N. OV-17 and the mixed column, OV-17 + QF-1, were applicable for the other chlordanes.

Column Chromatography

Separation of PCBs and t-N from the other chlordanes was necessary before gas chromatography, and Florisil chromatography permitted the separation and cleanup. Aldrin and the PCB components which disturb the t-N peak were eluted in the first hexane fraction (0-110 mL, discarded). t-N was recovered in the second hexane fraction (110-210 mL, Fr.A), together with p,p'- and o,p'-DDE, o,p'-DDT, the other PCB components which cause no

TABLE 1
GC Columns and Conditions

Liquid Phase	Support	Temp. (°C) Inj.& Det.	Temp. (°C) Column	N_2 Flow (mL/min)
2% Apiezon grease L	#1	240	200	80
2% OV-1 #2	#1	250	200	80
2% OV-17	Gaschrom Q,60-80 mesh	240	200	75
1.5% OV-17 + 1.95% QF-1	#1	250	200	80
0.5% OV-1 + 1.5% OV-210 #2	#1	250	180	80
2% OV-210	Shimalite W(AW-DMCS), 80-100 mesh	240	170	60
2.5% DEGS + 0.5% H_3PO_4	Shimalite W(AW-BW-DMCS),60-80 mesh	250	185	90
2% OV-275	#1	250	170	80

#1: Chromosorb W(AW-DMCS), 80-100 mesh
#2: glass column, 3 mm x 3 m, and others 3 mm x 2 m

TABLE 2
Retention Times of Chlordanes and Other Organochlorines (relative to aldrin)

Compound	Apiezon L	OV-1	OV-17	OV-17 + QF-1	OV-1 + OV-210	OV-210	DEGS + H_3PO_4	OV-275
oxychlordane	1.26	1.25	1.31	1.35	1.38	1.47	1.85	2.00
trans-chlordane	1.51	1.39	1.64	1.66	1.56	1.72	2.65	3.18
cis-chlordane	1.69	1.53	1.80	1.81	1.71	1.87	2.86	3.60
trans-nonachlor	1.71	1.61	1.73	1.71	1.65	1.69	2.62	2.96
cis-nonachlor	2.70	2.26	3.04	2.94	2.49	2.85	6.65	8.79
α-BHC	0.46	0.43	0.48	0.51	0.57	0.59	1.27	2.08
β-BHC	0.60	0.46	0.71	0.76	0.73	0.91	5.97	11.8
γ-BHC	0.57	0.52	0.62	0.66	0.70	0.76	1.96	3.43
δ-BHC	0.68	0.54	0.86	0.80	0.83	1.10	5.48	

TABLE 2 (continued)

heptachlor epoxide	1.23	1.21	1.43	1.51	1.54	1.84	2.77	4.49
dieldrin	1.97	1.71	2.20	2.33	2.34	2.92	4.14	5.34
endrin	2.88	1.96	2.76	2.84	2.67	3.53	4.72	
p,p'-DDE	2.22	1.73	2.23	2.12	1.84	2.00	3.43	3.30
p,p'-DDD	3.10	2.12	3.40	3.25	2.76	3.60	10.5	
p,p'-DDT	3.07	2.76	4.09	3.89	3.24	3.92	8.68	
o,p'-DDE	1.56	1.44	1.82	1.74	1.47	1.54	2.71	2.59
o,p'-DDT	2.20	2.25	3.32	2.99	2.37	2.57	4.72	
aldrin	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
(min)	(5.49)	(4.90)	(4.90)	(5.18)	(3.16)	(3.58)	(2.28)	(1.35)

TABLE 3
Recovery of Chlordanes from Fish and Shellfish

Sample	Added (ppm)	oxy-chlordane	trans-chlordane	cis-chlordane	trans-nonachlor	cis-nonachlor
gizzard shad (*Clupanodon punctatus*)	0.005	95 ± 2	95 ± 2	92 ± 3	84 ± 2	96 ± 2
	0.05	97.7 ± 0.2	97.0 ± 0.1	91.3 ± 0.7	93 ± 2	87.1 ± 0.3
short-necked clam (*Tapes Philippinarum*)	0.005	90 ± 1	87 ± 3	82 ± 2	84 ± 3	85 ± 1
	0.05	98.8 ± 0.2	95 ± 1	90 ± 2	89 ± 1	91 ± 1

Figures are average % recovery ± standard error (n=3).

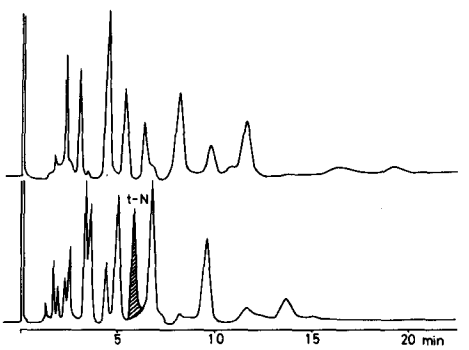
TABLE 4
Concentration of Chlordanes in Marine Fish and Shellfish (ppb on wet basis)

Sample	oxy-chlordane	trans-chlordane	cis-chlordane	trans-nonachlor#	cis-nonachlor	total chlordanes
goby (*Acanthogobius flavimanus*)	0.5 ± 0.02	1.5 ± 0.2	2.7 ± 0.2	7.1 ± 0.5 7.1 ± 0.8	6.1 ± 0.2	18
gray mullet (*Mugil cephalus*)	0.3 ± 0.02	4.0 ± 0.2	4.4 ± 0.4	3.6 ± 0.4 3.3 ± 0.6	1.2 ± 0.05	13
sea bass (*Lateolabrax japonicus*)	1.9 ± 0.3	11.3 ± 1.5	11.3 ± 0.6	12 ± 1 16 ± 1	2.1 ± 0.2	39
oyster (*Crassostrea gigas*)	1.1 ± 0.2	13.7 ± 0.9	15.4 ± 1.6	27 ± 0.9 31 ± 1	2.6 ± 0.3	60
blue mussel (*Mytilus edulis*)	n.d.	3.2 ± 0.4	5.3 ± 0.5	6.3 ± 0.6 5.2 ± 0.8	1.0 ± 0.1	16
thin-shelled surf clam (*Mactra veneriformis*)	n.d	0.9 ± 0.2	1.6 ± 0.3	1.6 ± 0.6 1.6 ± 0.2	0.5 ± 0.08	4.6
ark shell (*Scapharca subcrenata*)	0.3 ± 0.02	4.0 ± 0.1	6.6 ± 0.3	6.4 ± 0.2 6.1 ± 0.2	1.3 ± 0.06	19

Figures are average ± standard error (triplicated analyses).
Lower columns are values from SIM method.
n.d.: not detectable

interference, and a part of both p,p'-DDT and α-BHC (Fig. 2). The next 20% CH_2Cl_2-hexane fraction (150 mL, Fr.B) contained the other chlordanes, α-, β-, γ-, and δ-BHC, p,p'-DDD and p,p'-DDT. Frs.A and B applied to gas chromatographic quantification of the corresponding chlordanes. Heptachlor epoxide, dieldrin and endrin still remained on Florisil.

Chromatography required careful control of the adsorbent activity. The procedure is employed on the basis of several runs.



2% OV-1

First Hexane Fr. (discarded)

Fr.A (t-N Fr.)

Figure 2. Elution Pattern of PCBs
t-N quantification is free from interference, although PCB components are eluted in the two fractions.

Recovery and Comparison with SIM Quantification

Recovery tests were carried out by fortifying the samples with levels of 0.005 and 0.05 ppm each of these chlordanes. Recoveries of chlordanes ranged from 82 to 99% with high reproducibilities as Table 3 shows.

Field samples were collected from Tokyo Bay in 1978, and analyzed by the present method and partly by SIM (m/e 408.8, M-Cl). Chlordane peaks were separated enough to permit the quantification, and the t-N values from both methods compare quite well. Triplicated analyses of the field samples had relative standard error of 9.4% (average) on ppb levels (Table 4).

Minimum detectable levels of this method were approximately 0.2 and 0.1 ppb for cis-nonachlor and the other chlordanes, respectively.

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