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Residues of 4-Chloro-1-(2,4-dichlorophenoxy)-2-methoxybenzene(triclosan methyl) in Aquatic Biota

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Since 1975, we have monitored trace organochlorine compounds in fish and shellfish collected from Tokyo Bay, and attempted to identify unknown contaminants which have high sensitivity to electron capture detection (ECD). Recently, we found an unknown peak on the gas chromatogram of freshwater fish(*Carassius carassius*) samples collected at the Tama River pouring into Tokyo Bay. This peak was observed before that of dieldrin on a 2% OV-1 column. The unknown substance was extracted from the fish sample, cleaned up, and identified as methyl ether derivative of triclosan(triclosan methyl) by gas chromatography/mass spectrometry(GC/MS).

Triclosan(5-chloro-2-(2,4-dichlorophenoxy)phenol) is a broad spectrum antibacterial agent and has been widely used in varied products such as deodorant, surgical scrub preparations, and medicated cosmetics. Although, triclosan residues in rivers and industrial wastewaters have been found(Hites and Lopez-Avila 1979; Lopez-Avila and Hites 1980), the occurrence of triclosan methyl in aquatic biota has not been reported previously.

MATERIALS AND METHODS

A total of 14 samples(10 fish and four shellfish) was collected at 11 sampling locations in the Tama River and Tokyo Bay(Fig. 3) during the period from September to October, 1981.

GC/MS analysis was performed on a JEOL JMS D300 mass spectrometer equipped with a Hewlett-Packard 5710A gas chromatograph and JEOL JMA 2000 disc system under the following conditions: 0.2 x 180cm glass column packed with 2% silicone OV-1 on 80-100 mesh Gas-Chrom Q; EI, electron energy 70 eV; He 30 mL/min; oven temperature 200°C. High resolution measurements(resolution, ca. 5000): perfluorokerosene was used as a reference standard. Chemical ionization(CI) measurements were made at ca. 1 Torr. by use of CH_4 as a reagent gas.

ECD/GC analysis was carried out with three packed columns as shown in Table 2, and with a fused silica capillary column coated with silicone OV-1.

Proton(269.50MHz) and natural abundance ^{13}C(67.800MHz) NMR spectra were measured on a JEOL JNM-FX 270 spectrometer in $CDCl_3$ with tetramethyl silane as an internal reference.

Organic solvents were of pesticide grade or equivalent. Florisil column: activated Florisil(Floridin Co. 60-100 mesh, 20g, activated at 120°C for 15h before use) was packed in a glass column(2.2 x 30cm) with hexane. 4-Chloro-1-(2,4-dichlorophenoxy)-2-methoxybenzene (triclosan methyl), reference compound, was prepared from triclosan(Irgasan DP300, Ciba-Geigy) by methylation with diazomethane, and then purified by Florisil column chromatography. H and ^{13}C NMR data of triclosan methyl are as follows: H-NMR(δ, ppm); 3.809(3H,s), 6.671(1H,d, J=8.8Hz), 6.836(1H,d,J=8.6Hz), 6.900(1H,dd,J=2.2,8.6Hz), 6.978(1H,d,J=2.2Hz), 7.103(1H,dd,J=2.6,8.8Hz), 7.426(1H, d,J=2.6Hz). C-NMR(δ,ppm); 56.19(q), 113.58(d), 118.46 (d), 120.89(d), 121.13(d), 124.80(s), 127.70(d), 128.20 (s), 130.26(d), 130.35(s), 143.03(s), 151.40(s), 151.95 (s).

The sample(20g) was homogenized in a mixer(Ultra Turrax TP 18-10), dehydrated with anhydrous Na_2SO_4, and extracted with hexane(100, 80, 60mL). The combined hexane extract was evaporated to ca. 3mL in a Kuderna-Danish concentrator. The residue was dissolved in hexane(20mL) and partitioned with acetonitrile saturated with hexane (20mL x 4). The acetonitrile was poured into 3% Na_2SO_4 solution(350mL), and the solution was shaken with hexane (100, 80mL). The hexane extract was washed with 2% NaCl solution(50mL) and H_2O(50mL x 2), dried over anhydrous Na_2SO_4(10g), and concentrated to ca. 3mL. The concentrate was chromatographed on a Florisil column(20g). After washing the column with 20% dichloromethane in hexane(150mL), triclosan methyl was eluted with 50% dichloromethane in hexane(200mL). The fraction was concentrated to an appropriate volume, and determined by selected ion monitoring(SIM) mass spectrometry at m/z 302 and 304(EI, 25eV).

Pooled fish samples(*C. carassus*, 185g) which had been collected at Noborito, downstream along the Tama River, were extracted by the same method as described above, and then analyzed by GC/MS and ECD/GC.

RESULTS AND DISCUSSION

Fig. 2 shows the EI and CI mass spectra of unknown peak obtained from the freshwater fish(*C. carrassus*) sample. The fragmentation pattern of this substance in EI mode had the characteristics of Cl_3(m/z 302,M^+), and Cl_2(m/z 252,M-50). High resolution data(Table 1) of these peaks let to the tentative formula, and the chracteristic fragment ion at m/z 252 may be due to loss of CH_3Cl from the molecular ion. The mass spectrum was found to be similar to that of methyl ether derivative of triclosan(triclosan methyl) as described by Tulp and Hutzinger(1978). Then, an authentic specimen of triclosan methyl was prepared by methylation of triclosan. The unknown substance was identified as triclosan methyl by comparison with the reference compound in EI and CI mass spectra, and with retention times(Table 2). According to Tulp and Hutzinger(1978), the M-50 fragment ion was due to the chlorinated dibenzodioxin structure.

Fig. 1 shows the reconstructed ion current chromatogram from the fish sample. The occurrence of dieldrin, heptachlor epoxide, and musk xylene(artificial perfume) (Yamagishi et al. 1981), as shown in Fig. 1, were confirmed by comparing those of authentic compounds in mass spectra(EI mode) and the retention times on the fused silica capillary column.

Table 1. High resolution measurement of unknown compound

Observed mass(m/z)	Error(mmu)	Assignment
251.9771	2.6	$C_{12}H_6O_2{}^{35}Cl_2$
253.9731	1.5	$C_{12}H_6O_2{}^{35}Cl_1{}^{37}Cl_1$
301.9703	3.5	$C_{13}H_9O_2{}^{35}Cl_3$
303.9658	2.0	$C_{13}H_9O_2{}^{35}Cl_2{}^{37}Cl_1$
305.9653	4.3	$C_{13}H_9O_2{}^{35}Cl_1{}^{37}Cl_2$

Table 2. Relative retention times(aldrin=1.0) of unknown compound

Column	2% OV-1	2% OV-17	2% OV-210
Column temperature(°C)	220	215	200
Carrier gas(N_2, mL/min)	70	80	70
Relative retention times (aldrin=1.0)	1.35	2.04	1.85
aldrin(min)	(2.18)	(4.10)	(1.42)

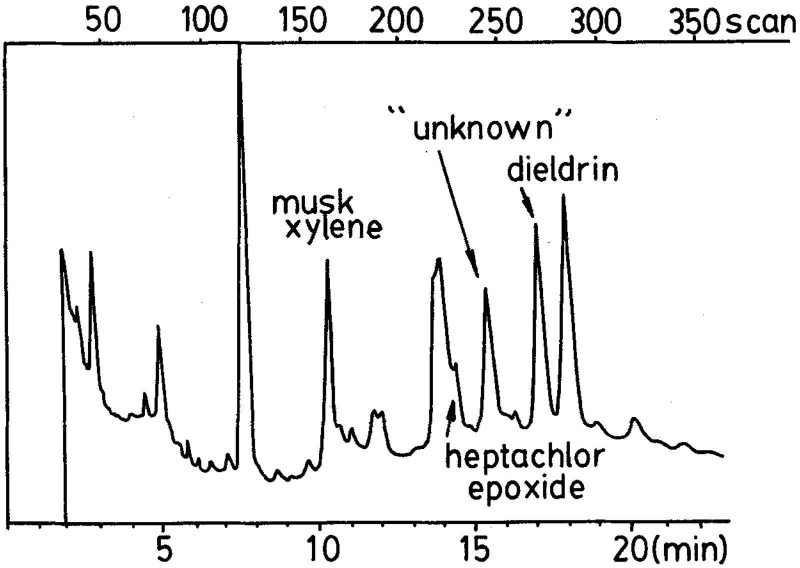

Fig. 1. Reconstructed ion current chromatogram from freshwater fish(*Carassius carassius*),OV-1(2%)

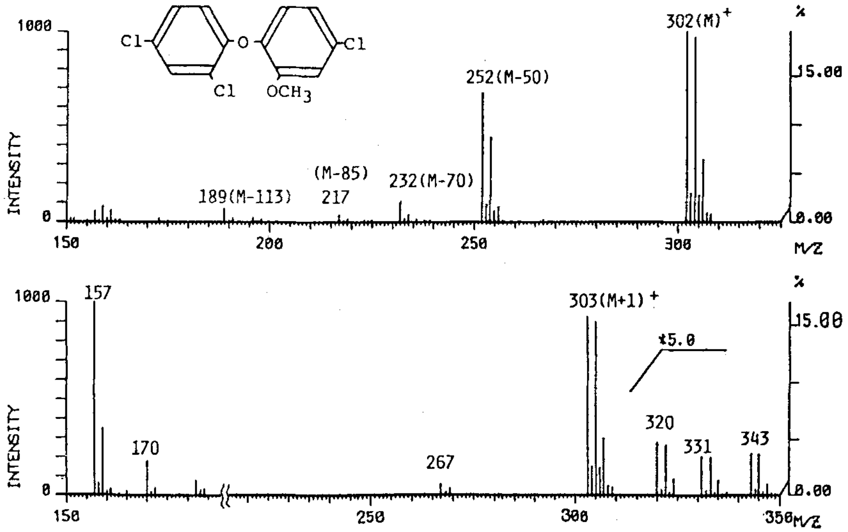

Fig. 2. EI(upper) and CI(lower) mass spectra of triclosan methyl in freshwater fish(*Carassius carassius*)

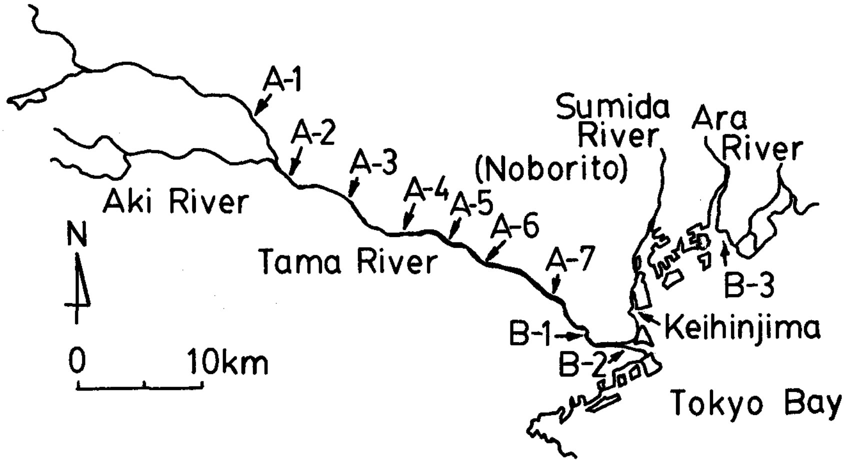

Fig. 3. Locations of sample collections

Table 3. Concentrations of triclosan methyl in fish samples from Tama River and Tokyo Bay (ppb on whole body basis)

Sample	Topmouth gudgeon(*P. parva*)							Goby(*A. flavimanus*)		
Locations	A-1	A-2	A-3	A-4	A-5	A-6	A-7	B-1	B-2	B-3
(ppb)	1	7	6	14	19	28	38	2	1	nd

* nd < 1 ppb

The *p*-value of triclosan methyl for hexane/acetonitrile was found to be 0.16 according to the method described by Bowman and Beroza(1965). This value indicates that hexane-acetonitrile partitioning was applicable to determination of the compound. The elution pattern of triclosan methyl with column chromatography on Florisil (Florisil, 20g) was examined with a dichloromethane-hexane solvent system. Consequently, after elution with 20% dichloromethane in hexane(150mL) to remove the interferences such as PCBs and chlordanes, triclosan methyl was eluted in the 50% dichloromethane in hexane (200mL) fraction. The determination of triclosan methyl was accomplished by SIM mass spectrometry at m/z 302 and 304. Its sensitivity was 5 pg at an ionization voltage of 25 eV. The analytical method was developed on the basis of the data described above. Recovery tests were carried out by fortifying the fish samples(*Lateolabrax japonicus*) with levels of 0.05 and 0.01 ppm each of triclosan methyl. Thus, recoveries(average ± S.D., in five determinations) were 86.0 ± 6.4% at 0.05 ppm, and 75.1 ± 3.8% at 0.01 ppm, respectively.

Table 3 shows the concentrations of triclosan methyl in aquatic biota collected from the Tama River and Tokyo Bay. The sampling locations are shown in Fig. 3. Triclosan methyl residue was found in 13 of 14 samples; detected in all freshwater fish(topmouth gudgeon,*P. parva*), and ranged 1-38 ppb(mean 11.6 ppb) on whole body basis. The concentrations in these fish samples increased downstream. The concentration in goby fish(*A. flavimanus*) in the estury of the river were relatively low, as shown in Table 3. Triclosan methyl residue was also found in shellfish taken from Keihinjima along Tokyo Bay(Fig. 3), and the concentrations were as follows: short-necked clam(*Tapes philippinarum*) 3 ppb, thin-shelled surf clam (*Mactra veneriformis*) 5 ppb, oyster(*Rassostrea gigas*) 13 ppb, blue mussel(*Mytilus edulis*) 20 ppb.

Pentachloroanisole was already known to be formed from pentachlorophenol by biological methylation(Cserjesi and Johnson 1972; Suzuki 1978). In addition , both halogenated phenols and the corresponding anisoles were identified in oysters collected at Keihinjima along Tokyo Bay (Miyazaki et al. 1981). We assume that triclosan methyl detection was due to biological methylation of triclosan on the basis of this information.

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