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Pollution Due to Volatile Halocarbon Compounds in Biota

Masayuki Gotoh,[1] Yoshiko Sekitani,[2] Teruyo Aramaki,[2] Haruo Kobayashi,[2] Keiki Ogino,[2] and Tatsuya Hobara[2]

[1]Department of Environmental Science, Ube College, 5-40 Bunkyo-cho, Ube 755, Japan and [2]Department of Public Health, Yamaguchi University School of Medicine, 1144 Kogushi, Ube 755, Japan

In recent years, volatile halocarbon compounds (VHCs) in drinking water have elicited increasing social concern and health problems (Rook 1974; Johnson et al. 1982). Further, it was reported that carcinogenic and/or mutagenic effects have been induced in animals by several VHCs (IARC 1979).

These substances were also detected in biota, sediment, and human food. Several methods were developed for the determination of VHCs in these types of samples. In each case, VHCs were eventually measured by gas chromatography. One of the pretreatment techniques involves the fairly simple procedure where samples are extracted with isooctane and subsequently isolated by micro florisil column (Daft 1989). However, this method is susceptible to low recovery. Ferrario et al.(1985) reported that ecosystems in Lake Pontchartrain were polluted based on the fact that VHCs were detected by the purge and trap method, using a pretreatment method slightly different from the ones mentioned above. Nevertheless, no report about an evaluation of the amount of pollutants in biota as human food was found.

In this report, the substantially improved Daft method for VHC analysis was applied to environmental biota and sediments, and an attempt was made to clarify the cause of pollution due to VHCs in biota. Furthermore, we found several interesting phenomena concerning the movement of VHCs in biota.

MATERIALS AND METHODS

Oyster(*Crassostrea gigas*), clam(*Tapes japonica*), and sediment samples were collected on five occasions from October to November 1990, at the Ariho and the Yoshinaga River(Figure 1). The Ariho River, which passes through an industrial area, is about 30 km in length. Towards the mouth and at the middle flow of the river, there are integrated circuits and dry cleaning product factories, which use 1,1,1-trichloroethane or tetrachloroethylene.

Send reprint requests to M.Gotoh at the above address.

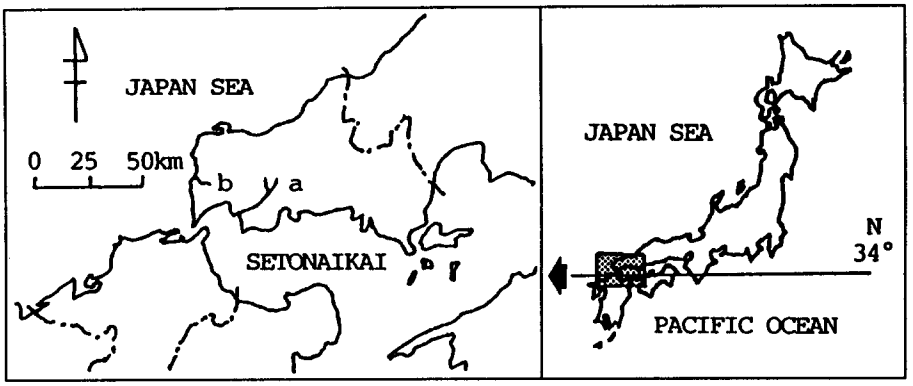

Figure 1. Location of the Ariho(a) and the Yoshinaga River(b).

The Yoshinaga River served as a control, which passes through a non-industrial area, is a short stream of about 10 km in length, and factories using VHCs are not present in the drainage area. Sampling of biota, sediment, and sea water in both rivers and the adjacent sea area were done at the same time. The clams were about 35 mm in size and about 1 g in wet weight of flesh. That of oysters were about 70 mm and 2 g. All samples were immediately packed in an ice box, and then kept at -5℃ until analysis.

We analyzed for eight VHCs:chloroform($CHCl_3$), dichlorobromomethane ($CHCl_2Br$),chlorodibromomethane($CHClBr_2$), bromoform ($CHBr_3$), carbon tetrachloride(CCl_4), 1,1,1-trichloroethane(MC), trichloroethylene (TCE) and tetrachloroethylene(PCE). The slightly modified version of micro column cleanup method (Daft 1989) was used to analyze biota and sediment samples. Sea water samples were extracted directly with solvent. The procedure of the analysis is as follows: 10 g of frozen body tissue (oyster and clam) were accurately weighed and crushed by mortar and pestle, sediments that were already measured contained water, and weighed exactly 10g in dry weight under wet conditions. Each of them was successively added to 10 mL of deionized water and 10 mL of n-hexane. Samples were extracted using a shaker(40 mm of width, 300 cycles/min x 30 min), and then centrifuged at 1800 x g for 10 min. Hexane phases were cleaned up by micro column ; more specifically, a micro florisil column was used for biota, and a micro silica column for sediments (Waters Associates,Inc.,Massachusetts). The first 2 mL of the effluent from the micro column was discarded and the following 2-mL volumes of the effluent were analyzed. 500 mL of sea water were extracted directly by two extractions with each 5 mL n-hexane. A 4-μL aliquot of the hexane phase was injected into a gas chromatograph(GC) equipped with a ^{63}Ni electron capture detector(ECD). The GC features and settings were as follows : Shimadzu GC-7AG, 3 m×3 mm(id) glass column with 20% silicone DC-550 Chromosorb W AW DMCS 60-80 mesh, N_2 carrier gas at 50 mL/min, injector and detector temperatures at 200℃ and column temperature at 80℃.

Moreover, for the measurement of the recovery tests, 0.1 mL of methanol, which was mixed with eight VHCs, was spiked into biota and sediment samples from the Yoshinaga River (which contained no detectable VHCs).

RESULTS AND DISCUSSION

The results of the recoveries were obtained on four experiments and were presented on Table 1. The mean recoveries were from 71.5 to 99.5% in oyster and clam, and from 91.3 to 100.0% in sediment. Daft(1989) performed analysis for 22 fumigants including VHCs in

Table 1. Recoveries of VHCs from biota and sediment[a]

Sample	$CHCl_3$		$CHCl_2Br$		$CHClBr_2$		$CHBr_3$	
	Added[b] μg	Recovery[c] %	Added[b] μg	Recovery[c] %	Added[b] μg	Recovery[c] %	Added[b] μg	Recovery[c] %
Oyster	0.2	80.0±3.8	0.05	86.2±2.3	0.08	93.8±2.5	0.4	96.3±2.9
	2	80.8±2.5	0.5	81.7±2.2	0.8	90.5±2.4	4	81.5±1.9
	20	86.7±1.6	5	82.3±1.8	8	88.2±1.5	40	80.0±1.7
Clam	0.2	80.8±4.4	0.05	87.2±2.9	0.08	82.2±3.4	0.4	80.3±1.9
	2	78.0±3.2	0.5	79.5±3.0	0.8	75.7±2.8	4	71.5±1.6
	20	81.5±2.7	5	78.7±3.0	8	80.3±2.4	40	78.3±1.4
Sediment	0.2	91.3±1.5	0.05	99.2±0.8	0.08	98.2±1.3	0.4	99.7±0.5
	2	92.8±2.0	0.5	96.0±1.7	0.8	99.0±1.1	4	98.0±1.4
	20	95.8±1.5	5	92.3±1.9	8	95.7±1.4	40	93.7±1.4
L.C.(ng/g)[d]		0.5		0.1		0.1		0.5

Sample	CCl_4		MC		TCE		PCE	
	Added[b] μg	Recovery[c] %	Added[b] μg	Recovery[c] %	Added[b] μg	Recovery[c] %	Added[b] μg	Recovery[c] %
Oyster	0.01	95.7±2.2	0.08	94.0±1.4	0.3	97.8±2.3	0.08	99.5±0.8
	0.1	90.8±2.0	0.8	94.5±1.0	3	98.0±1.3	0.8	98.7±0.5
	1	95.5±1.5	8	96.3±1.4	30	98.2±1.2	8	98.2±0.8
Clam	0.01	89.7±3.0	0.08	89.7±2.2	0.3	97.2±2.7	0.08	99.3±1.0
	0.1	90.5±1.5	0.8	87.2±1.8	3	88.3±1.0	0.8	99.2±0.8
	1	86.7±1.9	8	81.5±1.9	30	87.7±1.6	8	98.7±0.5
Sediment	0.01	98.2±1.2	0.08	98.3±1.4	0.3	97.8±1.2	0.08	100.0±0.6
	0.1	98.0±1.4	0.8	97.7±1.2	3	98.5±1.0	0.8	99.8±0.4
	1	99.3±0.8	8	99.0±0.9	30	99.3±0.8	8	99.8±0.4
L.C.(ng/g)[d]		0.01		0.1		0.5		0.1

[a] $CHCl_3$=chloroform; $CHCl_2Br$=dichlorobromomethane; $CHClBr_2$=chlorodibromomethane; $CHBr_3$=bromoform; CCl_4=carbon tetrachloride; MC=1,1,1-trichloroethane;TCE=trichloroethylene; PCE=tetrachloroethylene.

[b] Amount in 10g of raw biota or sediment as dry base.

[c] Recoveries represent the mean±SD of four experiments.

[d] L.C.=quantitated lowest concentration.

549 food samples, and obtained 55% of the mean recoveries. In our study, Daft method for VHC analysis was substantially improved. The quantitated lowest concentrations are also shown in Table 1. In addition, the gas chromatograms of blanks had not shown any disturbance peaks in analysis.

The concentrations of the eight VHCs in samples from both rivers are shown in Table 2. VHCs in sea water were not detected, except for trace of $CHBr_3$ in the brackish area of the Ariho River. $CHCl_3$, CCl_4, MC and PCE were detected in oyster, clam and sediment in the Ariho River. Thus, in a certain mouth of a river, the contamination by VHCs occurred. To compare with Ferrario's report(1985), the same kinds of compounds were detected. However, the concentrations in these data were all lower than those of Ferrario et al. These differences are caused by the differences in population, industrial or commercial activity and temperature at the sampling sites. MC, PCE and other VHCs were not detected in the Yoshinaga River. MC and PCE in biota and sediment in the Ariho River might have been related to pollution from the factories. However, $CHCl_3$ and CCl_4 in samples were detected in the Ariho River,where no factory uses these compounds. A possible reason is that $CHCl_3$ and CCl_4 are produced through biotransformation in biota or surrounding organisms because there are some reports that $CHCl_3$ is produced from other compounds in human or animals (Soucek et al. 1960; Fowler 1969). At the present time, there are no reports concerning VHC metabolism by organisms on the ocean bed. Further research is needed to clarify the biotransformation of VHCs in environmental subjects and experimental animals.

Table 2. VHCs mean concentration (ng/g) in biota and sediment

Sample	$CHCl_3$	$CHCl_2Br$	$CHClBr_2$	$CHBr_3$	CCl_4	M C	TCE	PCE
Ariho River								
Oyster[a]	6.5±2.1	ND	ND	ND	0.26±0.07	0.6±0.3	ND	0.6±0.2
Clam[a]	6.7±0.5	ND	ND	ND	0.54±0.05	1.8±0.3	ND	0.3±0.1
Sediment[b]	1.2±0.3	ND	ND	ND	0.07±0.01	0.4±0.1	ND	TR
Sea water[c]	ND	ND	ND	TR	ND	ND	ND	ND
Yoshinaga River								
Oyster[a]	ND	TR	ND	ND	TR	ND	ND	TR
Clam[a]	ND	TR	ND	ND	TR	ND	ND	TR
Sediment[b]	ND	ND	ND	ND	ND	ND	ND	ND
Sea water[c]	ND	ND	ND	ND	ND	ND	ND	ND

[a]Concentration is expressed as wet weight.
[b]Concentration is expressed as dry weight.
[c]Concentration is indicated with ng/L.
ND=not detected; TR=trace. Figures indicate the mean±SD. n=5.

About the processes of pollution due to VHCs in biota, it is believed that either bioconcentration from polluted sea water or sediment, or that bioaccumulation through benthic or planktonic-nektonic food webs occurred. In this study, no VHC was detected in sea water with our present method, but the sea water might

include a slight volume of VHCs. Therefore, the pollution of VHCs in biota at the Ariho River may accumulate over a long period of time. It is necessary that a more sensitive method is utilyzed to analyze sea water.

Customarily, Japanese people soak clams in sea water for 24 hr in order to avoid chewing on fine sand present in the bivalves before cooking. The oysters and clams are then boiled.

Table 3. Decreased concentration (ng/g) of VHCs in biota by settling 1 d in sea water and subsequent boiling

Sample	$CHCl_3$	CCl_4	M C	PCE
Oyster				
Initial	6.5±2.1	0.26±0.07	0.6±0.3	0.6±0.2
After 24hr	ND	ND	ND	0.4±0.1*
After 24hr+boiling	ND	ND	ND	0.2±0.1**
Clam				
Initial	6.7±0.5	0.54±0.05	1.8±0.3	0.3±0.1
After 24hr	ND	ND	0.6±0.1**	0.2±0.1*
After 24hr+boiling	ND	ND	0.1±0.0**	0.1±0.1**

ND=not detected. Figures indicate the mean±SD.n=5.
**$p<0.01$, *$p<0.05$.

As a result, the changes in concentrations of four VHCs in biota were studied before and following immersion in sea water and subsequent boiling. As shown in Table 3, the concentrations of the four VHCs in biota were substantially decreased after a 24 hr soaking period and even further decreased after boiling. This phenomenon might have been caused by excretion or biotransformation of VHCs in biota. Moreover, after boiling, the differences of VHCs decrease were characterized by volatility. The decrease of PCE was small compared with those of $CHCl_3$ and CCl_4. Thus, chemical and physical properties of VHCs, such as boiling point, vapor pressure and partition coefficient(Sato et al. 1979), may explain the differences in the degrees of concentration in biota. We could suggest that excretion from biota was the main reason for the decrease in VHCs concentrations. However, there are no available data concerning the biotransformation of VHCs in biota. In future studies, we will investigate the details of metabolism, biotransformation, movement and othor factors of VHCs in biota.

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REFERENCES

Daft JL (1989) Determination of fumigants and related chemicals in fatty and nonfatty foods. J Agric Food Chem 37:560-564
Ferrario JB, Lawler GC, Deleon IR, Laseter JL (1985) Volatile or-

ganic pollutants in biota and sediments of Lake Pontchartrain. Bull Environ Contam Toxicol 34:246-255
Fowler JSL (1969) Carbon tetrachloride metabolism in the rabbit. Brit J Pharmacol 37:733-737
IARC(1979) IARC monographs on the evaluation of the carcinogenic risk of chemicals to humans. Some halogenated hydrocarbons, World Health Organization, Lyon
Johnson JD, Christman RF, Norwood DL, Millington DS(1982) Reaction products of aquatic humic substances with chlorine. Environ Health Perspect 46:63-71
Rook JJ(1974)Formation of haloforms during chlorination of natural waters. Water Treat Exam 23:234-243
Sato A, Nakajima T(1979) A structure-activity relationship of some chlorinated hydrocarbons. Arch Environ Health 34:69-75
Soucek B, Vlachova D (1960) Excretion of trichloroethylene metabolites in human urine. Brit J Ind Med 17:60-64

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