Bull. Environ. Contam. Toxicol. (1998) 60:74-80
© 1998 Springer-Verlag New York Inc.

Bulletin of Environmental Contamination and Toxicology

Pollution due to Trichloroacetic Acid in Clams (*Tapes japonica*) in an Estuary Adjacent to Industrial Areas

M. Gotoh,[1] T. Hobara,[2] H. Kobayashi,[2] M. Okuda[2]

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

Received: 30 May 1997/Accepted: 1 October 1997

We found that clam samples from an industrial area were polluted by volatile halocarbon compounds (VHC), e.g. tetrachloroethylene, chloroform, carbon tetrachloride and 1,1,1-trichloroethane (Gotoh et al. 1992). Moreover, in that report we pointed out the potency of biometabolism of VHC in clams from the phenomena that VHC concentrations of the clam samples decreased remarkably during incubation for 24h in sea water. Generally, in human and several experimental animals, it is well known that inhaled trichloroethylene (TRI) or tetrachloroethylene (PCE) are metabolized to trichloroacetic acid (TCA) and trichloroethanol (TCE) (Daniel 1963; Hobara et al. 1986). Nevertheless, there are extremely few reports about movement during metabolism or accumulation of TRI and PCE in biota such as clams.

In this report, We developed a method for analysis of TCA and TCE in clams, sediment, and sea water samples, and applied our present method to each sample from two industrial areas in order to find TCA and TCE pollution. Furthermore, we tried to know whether, in clams, TRI and PCE could be metabolized to TCA and/or TCE.

MATERIALS AND METHODS

Clams (*Tapes japonica*), sediment and sea water samples were collected on five occasions from September to November in both 1995 and 1996, at the Ariho, Koe and Okita Rivers (Fig.1), respectively. The Ariho River, which passes through an industrial area, is about 30 km in length. Towards the estuary and at the middle flow of the river, there are integrated circuit and dry cleaning product factories, which use l,l,l-trichloroethane or PCE. The Koe River, which passes through a small industrial area, is about 12 km in length. There are some hospitals using TCA at the mouth of the river, but no factories using VHC. The Okita River, which passes through a non-industrial area served as a control. It is a short stream of about 10 km in length, and receives no VHC, TCA or TCE pollution. Sampling of clams, sediment and sea water in the three rivers were done at the same time. The clams were about 35 mm in size (about 1 g in wet weight of flesh). All samples were packed in ice, and then immediately analyzed for TCA, TCE, TRI

Correspondence to: M. Gotoh

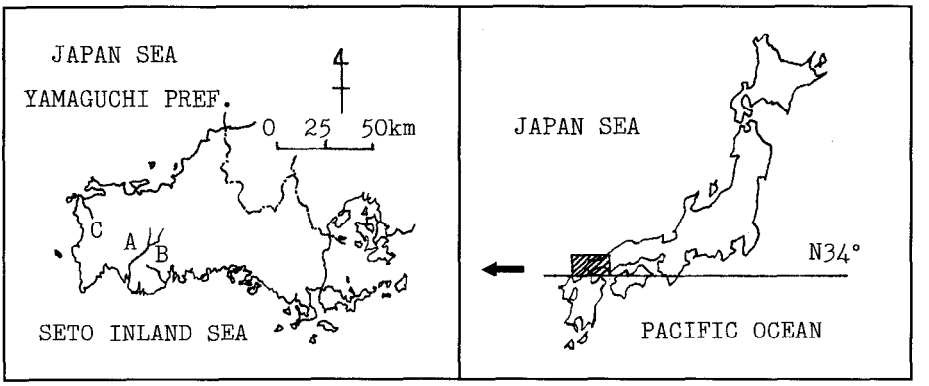

Figure 1. Location of the Ariho River (A), the Koe River (B) and the Okita River(C).

and PCE. TCA and TCE of clams, sediment and sea water samples were analyzed with a slightly modified version of the method of Humbert and Fernández (Humbert and Fernández 1976), which was applied to human serum and urine samples. The procedure of analysis was as follows : Clam samples were homogenized for 5 min at 15,000 rpm with a homogenizer. 5 g of clam tissue was accurately weighed. Sediment samples were already measured containing water, and weighed 5 g of dry weight under wet conditions. Sea water samples weighed 5 g. Each of them was successively added to 5 mL of distilled water, 2 mL of hydrochloric acid, and 10 mL of 1,3-dibromopropane (internal standard)1 µL/L diethyl ether. Samples were extracted using a shaker (40 mm of width, 300 cycles/min X 10 min), and then centrifuged at 1,800 X g for 5 min. 2 mL of ether phases were rapidly added in about 10 mg of 3-methyl-1-p-tryltriazene, and TCA was methylated for 10 min. 1 mL of n-hexane was added. A2 µL aliquot of the extract was injected into a gas chromatograph (GC) equipped with a ^{63}Ni electron capture detector (ECD). The GC features and settings were as follows : Hitachi GC 263-50, 2 m X 3.2 mm id glass column with 5 % silicone OV-17 GaschromeQ 100-120 mesh, N_2 carrier gas at 40 mL/min, injector and detector temperatures at 180 °C and column temperature at 110 °C. Moreover, for the measurement of the recovery tests, 1 ml of TCA or TCE standard solution was spiked into 5 g of clam tissue, sediment and sea water samples from the Okita River in 1995 (which contained no detectable TCA and TCE), whose final TCA and TCE concentrations in each sample were made of 30, 80, 150, and 300 µg/kg respectively. Each of them was added to 4 mL of distilled water, and the above mentioned treatment was successively done. These tests were done five times.

The qualities of TCA or TCE in the field samples detected by GC(ECD) confirmed by gas chromatograph/mass spectrometry (GC/MS). GC/MS conditions were as follows: Hewlett Packard HP6890 Series MSD, DB-1701 60 m×0.25 mm id× 0.25 µm df fused silica column, He carrier gas at 1.0 mL/min (15.6 psi at 35 °C), inlet mode by pulsed splitless (pulse 40.0 psi 1.70 min), injector temperature at

260 °C, column temperature at 35 °C(2.0 min) - 20 °C/min - 260 °C(10.0 min), interface temperature at 260 °C, ion source temperature at 160 °C, ionized method by electron impact at 70 eV scan range of 35-350 amu., scan speed at 1 s/scan, injection by HP6890 Series Autosampler, injected volume of 5 µL.

The analysis of TRI and PCE in specimens was done according to the method of Gotoh et al.(Gotoh et a1.1992). In short, extraction with n-hexane was performed for clams, sediment and sea water samples. The extract was subsequently analyzed by GC. GC features and settings were as follows : Shimadzu GC 7AG equipped with a ^{63}Ni ECD, 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 °C and column temperature at 80 °C.

A preliminary *in vitro* study concerning metabolism of TRI or PCE was conducted at mentioned below. 5 g of homogenized clam's mid-gut gland (no detectable TRI, PCE, TCA and TCE), which was sampled from the Okita River in 1997, was put in a 50mL glass tube with glass stopper. 1 mL of the TRI or PCE sea water solution (which was prepared like that of 10 µL of TRI or PCE standard solution put into 50 mL of sea water in a glass tube with glass stopper and after 10 min shaking) was added to the clam's gonad. TCA, TCE, TRI and PCE were measured before and after incubation for 6 h at 25 °C. These experiments were done five times. In addition, Student's t test was used to determine statistical significance.

RESULTS AND DISCUSSION

The results of the TCA and TCE recoveries are presented in Table 1. The mean recoveries were from 81.6 to 90.3 % of TCA and from 94.3 to 97.2 % of TCE in clams, from 90.5 to 91.8 % of TCA and from 98.3 to 99.3 % of TCE in sediment, and from 99.5 to 99.7 % of TCA and from 99.7 to 99.9 % of TCE in sea water. The recoveries were satisfactory in all samples, and standard curves of TCA and TCE are shown linearly below 300 µg/kg of each sample. The quantitated lowest concentrations are 1µg/kg both of TCA and TCE. In addition, the gas chromatograms of blank tests had not shown any disturbing peaks in analysis. Thus, we could get a near satisfactory analysis of TCA and TCE in clams, sediment and sea water samples.

The concentrations of TCA, TCE, TRI and PCE in clam samples from three rivers in 1995 are shown in Table 2. In clams from locations A and B, which are industrial areas, TCA was detected at 21±2 µg/kg and 50±6 µg/kg (mean±SD), but TCE was not detected in clams from these locations. The qualities of TCA were confirmed by GC/MS. Trichloroacetic acid methyl ester appeared at 9.2 min of retention time in GC/MS analysis. TCA and TCE in sediment and sea water samples from three locations in 1995 and 1996 were all below detectable levels. TCA concentrations in clam samples in 1996 were at 27±3 µg/kg in location A and

at 69±4 μg/kg in location B. This data shows an increase of about 30-40 % over the previous year, and was statistically significant ($p<0.01$). This fact may suggest the below two reasons.

Table 1. Recoveries of TCA and TCE from clams, sediment and sea water.

Sample		TCA		TCE
	Concentration (μg/kg)	Recovery [a] (%)	Concentration (μg/kg)	Recovery [a] (%)
Clams [b]	30	87.9 ± 2.3	30	95.0 ± 1.4
	80	88.0 ± 2.5	80	95.0 ± 1.7
	150	90.3 ± 3.0	150	97.2 ± 1.2
	300	81.6 ± 3.5	300	94.3 ± 2.6
Sediment [c]	30	91.5 ± 2.2	30	99.3 ± 0.8
	80	91.8 ± 2.6	80	99.0 ± 0.9
	150	90.5 ± 2.2	150	99.2 ± 0.8
	300	90.6 ± 3.1	300	98.3 ± 1.1
Sea Water	30	99.5 ± 0.4	30	99.9 ± 0.0
	80	99.7 ± 0.4	80	99.9 ± 0.1
	150	99.6 ± 0.3	150	99.7 ± 0.2
	300	99.5 ± 0.4	300	99.7 ± 0.2
L.C.(μg/kg)[d]	1		1	

a) Recoveries represent the mean ± SD of five experiments.
b) Concentration was expressed as raw weight.
c) Concentration was expressed as dry base.
d) L.C. = quantitated lowest concentration. unit = μg/kg.

Table 2. The concentrations (μg/kg) of TCA, TCE, TRI, and PCE in clam samples which were sampled in locations A, B and C in 1995.

Sampling point	A	B	C
TCA	21 ± 2	50 ± 6	ND
TCE	ND	ND	ND
TRI	ND	ND	ND
PCE	0.6 ± 0.2	TR	ND

ND : not detected (TCA<1, TCE<1, TRI<0.5, PCE<0.1μg/kg).
TR : trace.
Figures indicate the mean ± SD. n=5.

First is that bioconcentration of TCA in clams has occurred. The existence of TCA

in clams from locations A and B might have been related to TCA pollution induced by the disinfection by-products of water treatment (Minear and Amy 1996), medical reagents or herbicides. There are several waste water treatment facilities near locations A and B, and some hospitals near location B. About the processes of pollution due to TCA in clams, 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 TCA was detected in sea water with our present method, but the sea water might include a slight volume of TCA. The pollution of TCA in clams at locations A and B may accumulate over a long period of time, or a temporary pollution may have occurred. It is necessary that a more sensitive method is utilyzed to analyze sea water. In addition, in order to ascertain the reason for water pollution of TCA, TCA analysis of waste water from these facilities should be done.

Table 3. The concentrations of TCA and TCE in clams' mid-gut gland on a 6h incubation at 25°C after TRI or PCE was placed.

	Concentration [a)]	
	Initial	After 6h
TRI	38.5 ± 1.2	38.1 ± 1.5
TCA	ND	TR
TCE	ND	ND
PCE	39.2 ± 2.7	38.7 ± 2.5
TCA	ND	TR
TCE	ND	ND

a) : unit ; TRI and PCE = mg/kg, TCA and TCE = μg/kg.
ND : not detected (TCA, TCE < 1μg/kg).
TR : trace
Figures indicate the mean ± SD. n=5.

Second is that TCA is produced through biometabolism in clams or surrounding organisms. There are many reports stating that TCA is produced from TRI and PCE as metabolite in human and experimental animals (Daniel 1963; Green and Prout 1985; Hobara et al. 1986). TRI and PCE are metabolized to tri- or tetra-chloroethylene oxide as intermediate metabolites, and to TCA and TCE as final metabolites mainly by liver. At the present time, there are no reports concerning TRI or PCE metabolism by organisms on the ocean bed. We subsequently tried to determine whether in clams TRI and PCE could be metabolized to TCA and/or TCE or not, by a preliminary *in vitro* experiment. We showed the results of biometabolism of TRI and PCE in Table 3. TRI and PCE concentrations (mean±SD) in clam's mid-gut gland were at 38.5±1.2 mg/kg and at 39.2±2.7 mg/kg respectively. TCA were detected at trace and TCE were not detected in

clam's mid-gut gland after 6h incubation, in the case of both TRI and PCE. The concentrations of TRI or PCE after incubation for 6h appeared to slightly decrease however the changes were not statistically significant. Consequently, an ability to biometabolise TRI and PCE to TCA and/or TCE was not negative. There are almost no available data concerning biometabolism of TRI and PCE in clams. Further research is needed to clarify the biometabolism of TRI and PCE in environmental subjects and experimental animals.

It is shown in table 2 that the presence of TCA in clam samples in location B was larger than in location A in spite of almost no detection of PCE. We suppose from this fact that bioconcentration is a plausible reason rather than biometabolism. The above mentioned, water pollution by TCA in these industrial areas may have occurred, and this would perhaps account for the presence of TCA in clam tissue. If this explanation is applicable, the monitoring of TCA using clams should be one means to facilitate obtaining data on water pollution due to TCA. In addition, the hepatic injury by TCA in human and several experimental animals was reported (Pamell et al. 1988; Larson and Bull 1992). It is accordingly very important to determine TCA movement in clams because Japanese people have traditionally always eaten clams. Thus we found pollution due to TCA in clam samples from industrial areas, but the reason for TCA's presence was unclear. In future studies, we will investigate the details of bioconcentration and biometabolism concerning TCA in clams.

Acknowledgments. We thank Mr. Yoshihiro Ohmiya for his help with TCA and TCE analysis, and Mr. James Fryxell and Mr. Steve Sidwell for their advice and assistance in the experiments. This work was suported in part by Grants in Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan.

REFERENCES

Daniel JW (1963) The metabolism of ^{36}Cl-labelled trichloroethylene and tetrachloroethylene in rat. Biochem Pharmacol 12:795-802

Gotoh M, Sekitani Y Aramaki T, Kobayashi H, Ogino K, Hobara T (1992) Pollution due to volatile halocarbon compounds in biota.Bull Environ Contam Toxico1 49:186-191

Green T, Prout MS (1985) Species differences in response to trichloroethylene.II biotransformation in rats and mice. Toxicol Appl Pharmacol 79:401-411

Hobara T, Kawamoto T, Sakai K (1986) Extrahepatic organs metabolism of inhalated trichloroethylene. Toxicology 41:289-303

Humbert BE, Fernández JG (1976) Simultaneous determination of trichloroacetic acid and trichloroethanol by gas chromatography. Int Arch Occup Environ Health 36:235-241

Larson JL, Bull RJ (1992) Metabolism and lipoperoxidative activity of trichloroacetate and dichloroacetate in rats and mice.Toxicol Appl Pharmacol 115:268-277

Minear RA, Amy GL (1996) Disinfection by-products in water treatment. Lewis Publishers, Florida

Parnell MJ, Exon JH, Koller L.D (1988) Assessment of hepatic initiation-promotion properties of trichloroacetic acid. Arch Environ Contam Toxicol 17:429-436