

## DETERMINATION OF TRICLOSAN AND ITS RELATED COMPOUNDS IN AQUATIC ENVIRONMENT

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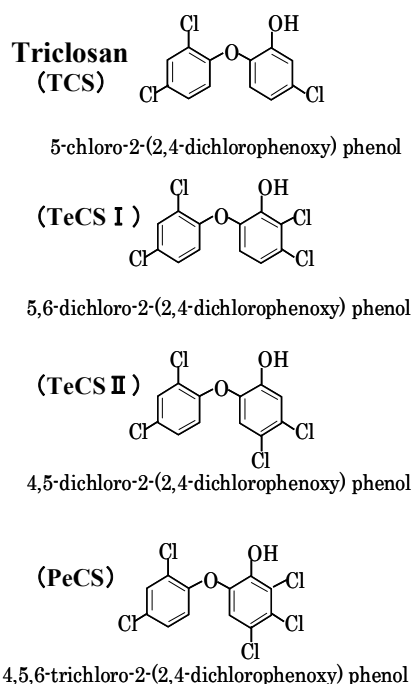
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### Abstract

5-chloro-2-[2,4-dichlorophenoxy]phenol (triclosan: TCS) is an antimicrobial agent used widely for medical and consumer care products and has attracted concerns about its impact on organisms living in receiving rivers of medical and domestic wastewaters. In this study, we investigated the distribution and behavior of TCS and its chlorinated derivatives [5,6-dichloro-2-[2,4-dichloro-phenoxy]-phenol (TeCS I), 4,5-dichloro-2-[2,4-dichloro-phenoxy]-phenol (TeCS II) and 4,5,6-trichloro-2-[2,4-dichloro-phenoxy]-phenol (PeCS)] which might be unintentionally formed during the chlorine bleaching and sterilizing processes. A sensitive and simple method for analysis of TCS and its related compounds for water sample was developed. The recoveries of the method resulted in 87-94% for dissolved form, and 78-89% for particles-bound form. TCS in water samples (sum of dissolved and particles-adsorbed forms) were detected in the range of 113-296 ng/l for effluent from wastewater treatment plant and 0.16-205 ng/l for river water. Sum of chlorinated derivatives were detected in the range of 6.6-9.9 ng/l and 0.13-5.9 ng/l, respectively. The percentage of particles-bound form in whole water samples were 2.3-22% for TCS, 5.1-35% for TeCS I, 6.9-59% for TeCS II and 5.6-65% for PeCS.

### Introduction

There is a growing concern about the occurrence and fate of pharmaceuticals and personal care products (PPCPs) in the environment. 5-chloro-2-[2,4-dichlorophenoxy]phenol (triclosan: TCS) is one of PPCPs, and has been widely used as an antimicrobial agent for many household and consumer care products such as medical soap, toothpastes, and deodorants. On the other hand, it has been suggested that TCS may be toxic to aquatic organisms<sup>1)</sup> and recently it has been reported that exposure to low levels of TCS can disrupt the thyroid hormone-associated gene expression and can alter the rate of thyroid hormone-mediated postembryonic anuran development<sup>2)</sup>. Consequently, there is concern about its impact on organisms living in receiving rivers of domestic wastewater and municipal wastewater treatment effluent. Chlorinated derivatives of TCS, namely TeCS I, TeCS II, and PeCS as shown in Fig.1, which were not contained in TCS preparation, have been indicated to be unintentionally formed during the chlorine bleaching and sterilizing processes<sup>3)</sup>. As it has been reported that these compounds can easily be converted to polychlorinated dibenzo-*p*-dioxins by heating or exposure to sunlight and UV irradiation<sup>4)</sup>, they have needed to be monitored as environmental pollutants. Environmental occurrence of TCS in river, lake, and effluent from wastewater treatment plant has been reported in Europe and the United States, only a little information is available in Japan<sup>5) - 9)</sup>. Chlorinated derivatives probably due to difficulty in detection have not been measured, and their behavior in the aquatic environment has not been elucidated. The purpose of this study was to develop the simultaneous analytical methods for TCS and its chlorinated derivatives and to determine their distribution and behavior in the aquatic environment.



**Fig. 1. Chemical structures of triclosan and its chlorinated derivatives.**

### Materials and Methods

**Analytical standards** A native TCS and  $^{13}\text{C}_{12}$ -labeled TCS were purchased from Wako Pure Chemicals Co. (Osaka, Japan) and Wellington Laboratories (Ontario, Canada), respectively. Chlorinated derivatives of TCS were synthesized by Dr. Akio Kanetoshi, Hokkaido Institute of Public Health, Hokkaido, Japan, and they were provided by Dr. Tameo Okumura, Environmental Pollution Control Center, Osaka, Japan.

**Sample collections** Water samples were collected from 20 rivers over Japan. The effluent from wastewater treatment plant sample was collected from Matsuyama, Japan. These samples were collected during February 2006 to January 2007.

**Extraction and cleanup** Water sample was filtered through glass fiber filter using vacuum sucking filtration equipment in order to separate suspended solid (SS) and filtrate. To determine the dissolved form of the target compounds, 5 ng of  $^{13}\text{C}$ -TCS as internal standard was added to the filtrate, and pH of the solution was adjusted to 2-3 with hydrochloric acid prior to solid-phase extraction. The prepared water sample was passed through Oasis HLB SPE cartridge (Waters Co., MA, USA) or Strata-X SPE cartridge (Phenomenex Co., CA, USA) preconditioned with 5 ml each of dichloromethane, methanol, and distilled water. After passage of the prepared water sample, the cartridge was rinsed with 5 ml of 30% (v/v) methanol in distilled water as the first rinse and dried for no more than 1 hours and rinsed with 10 ml of 10% (v/v) dichloromethane in hexane as the second rinse. The target compounds were then eluted from the cartridge with 10 ml of dichloromethane. The dichloromethane eluent was replaced with hexane and evaporated under a gentle stream of  $\text{N}_2$  to a volume of approximately 200  $\mu\text{l}$  prior to derivatization. 25  $\mu\text{l}$  of methanol and 10  $\mu\text{l}$  of trimethylsilyl-diazomethane (TMSDA) (ca. 10% in n-Hexane) (Tokyo Chemical Industry Co., Tokyo, Japan) were added and the mixture solution was allowed to react for 12 hours at room temperature. Subsequently, the solution after reaction were purified with 44% sulfuric acid silica gel column, which prepared by filling column with 1g of Wakogel S-1 (Wako Pure Chemicals Co., Osaka, Japan) impregnated with 44% (w/w) sulfuric acid. After adding the cleaned and reacted solution to the column, the compounds were eluted with 30 ml of hexane. The hexane eluent was replaced with decane. 5 ng of  $^{13}\text{C}$ -TeCB was spiked to the solution as an internal standard (syringe spike) and evaporated to a volume of approximately 50  $\mu\text{l}$ . Then HRGC-LRMS analysis was performed. On the other hand, to determine SS-bound form of target compounds, the filter with residues was dried overnight and then cut into small pieces and put in the centrifuging tube. 5 ng of  $^{13}\text{C}$ -TCS and 30 ml of acetone were then added to the tube, and subjected to ultrasonic extraction for 10 min under acidic condition. The solution was centrifuged at 3000rpm for 10 minutes, and acetone solution was collected. Additional 30 ml of acetone was added into the residue, and the same operation was repeated more two times. The combined acetone solution was evaporated to a volume of 10 ml and mixed with 90 ml of distilled water. The solution was analyzed by the same analytical procedures as dissolved form, namely solid-phase extraction, derivatization, column cleanup and GC-MS analysis.

**GC-MS analysis** The cleaned-up sample was analyzed by splitless injection (2  $\mu\text{l}$ , 280°C) with a HP6890 gas chromatograph (Agilent Technology, USA) equipped with a HT8-PCB column (60 m length, 0.25-mm inner diameter, 0.25- $\mu\text{m}$  film thickness) coupled to a JMS-AM SUN low resolution mass spectrometer (JEOL, Japan) (electron impact ionization mode, 70 eV, 150  $\mu\text{A}$ , 250 °C). The GC column was initially held at 90°C for 1 min, increased to 180°C at a rate of 30°C/min, and then increased to 230°C at a rate of 7°C/min, and then increased to 280°C at a rate of 20°C/min, and finally held at 280°C for 6.5 min. Helium was used as a carrier gas (1.0ml/min). Two selected ions for each compound were monitored. These ion masses were 302/304 m/z for TCS, 338/336 m/z for TeCS I and TeCS II, and 372/370 m/z for PeCS, respectively. The internal standard was monitored at 314/316 m/z for  $^{13}\text{C}$ -TCS, and 302/304 m/z for  $^{13}\text{C}$ -TeCB.

### Results and Discussion

**Development of analytical methods** More sensitive and simple analytical method for water sample was developed on the basis of the method which is recommended by the Ministry of Environment, Japan, and reported by Okumura et al.<sup>10)</sup>. Three analytical steps were modified to improve procedures, 1) solid-phase extraction was applied instead of liquid-liquid extraction which is required large quantity of organic solvent. We

also attempted to eliminate methylated TCS, which was a possible degradation product of TCS by bacterial activities, by rinsing solid-phase cartridge with 5% dichloromethane in hexane after passing through a water sample. 2) TMSDA which was less explosive and less toxic was used instead of diazomethane. 3) A column chromatography with silica gel impregnated with sulfuric acid was introduced to remove efficiently interfering substances. Recovery test was carried out in order to evaluate the accuracy and precision of the developed analytical method. The results are shown in Table 1.

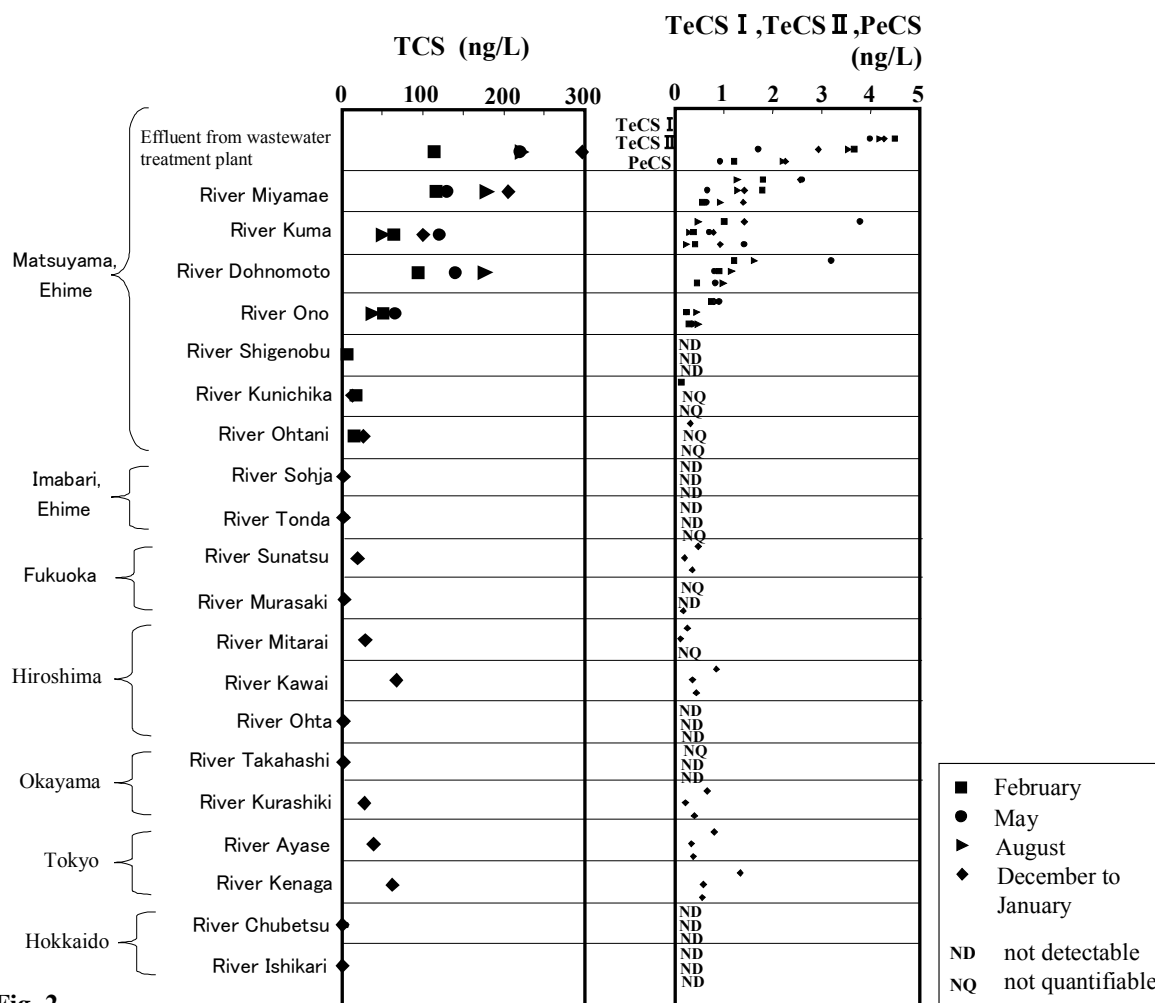
**Table 1.**  
**Recovery of TCS and its chlorinated derivatives with the present methods.**

| Compounds | Dissolved form (n=3) |      | Particles-bound form (n=3) |      |
|-----------|----------------------|------|----------------------------|------|
|           | % recovery           | % CV | % recovery                 | % CV |
| TCS       | 94                   | 3.4  | 89                         | 0.65 |
| TeCS I    | 91                   | 4.1  | 86                         | 1.8  |
| TeCS II   | 87                   | 1.4  | 81                         | 1.2  |
| PeCS      | 93                   | 6.6  | 78                         | 5.9  |

**Analysis of river water and effluent from wastewater treatment plant** The results are shown in Fig.2. Total amount of TCS in dissolved and particles-bound forms of water samples were detected in the range of 0.16-205 ng/l for river water, 113-296 ng/l for effluent from wastewater treatment plant. TCS concentrations detected in six water samples were more than 50 ng/l. Danish Environmental Protection Agency has recommended the predicted-no-effect-concentration (PNEC) of TCS as 50 ng/l, which is based on the value, no-observed-effect-concentration which is determined as 500 ng/l for freshwater aquatic organisms <sup>1)</sup>. It has been reported also that exposure to TCS concentrations as low as 150 ng/l result in a disruption of thyroid hormone-associated gene expression, then consequent interference about normal metamorphosis of an anuran <sup>2)</sup>. The observed TCS concentrations more than 150 ng/l in Rivers Miyamae and Dohnomoto may indicate a potential impact on river ecosystem.

That high concentration in River Miyamae might be attributed to effluent from wastewater treatment plant along the river. The River Dohnomoto, which does not have wastewater treatment plant along the river, might be contaminated by the direct input of domestic wastewater or water inflow from sewage treatment tank from individual houses. Detailed research about the source and route will be necessary. The results of recent studies in Japan show that TCS levels in effluent from 5 wastewater treatment plant of Tokyo were in the range of 26.6-330ng/l <sup>5)</sup>, and concentrations in river around Japan ranged 8-35ng/l <sup>6)</sup>. On the other hand, the concentrations in effluents from 7 wastewater treatment plant and in its 2 receiving rivers in Switzerland were in the range of 42-213 and 11-98 ng/l, respectively <sup>7)</sup>. The concentrations in effluent from 4 wastewater treatment plants and in eighty-five rivers around the United States ranged 200-2700 ng/l <sup>8)</sup>, and as median concentration of 140 ng/l <sup>9)</sup>, respectively. Our results, both for river and effluent, were within the range of these research results. On the present study, chlorinated derivatives of TCS (TeCS I, TeCS II, PeCS) were detected at the concentrations over the limit of quantification. The concentrations of TeCS I ranged 0.13-3.8 ng/l, 0.10-1.78 ng/l for TeCS II, 0.16-1.4 ng/l for PeCS in river water; 4.0-4.5 ng/l for TeCS I, 1.7-3.7 ng/l for TeCS II, 0.91-2.3 ng/l for PeCS in effluent from wastewater treatment plant. To date, the data about these compounds in river water have not been reported as far we are concerned. It is thought that those are significant data to understand about their behavior in aquatic environment. In the present study, TeCS I was detected in the highest concentration among higher chlorinated derivatives.

The form of TCS and its chlorinated derivatives was examined whether dissolved form or particles-bound form was dominant. The percentage of particles-bound form in water samples were 2.3-22% for TCS, 5.1-35% for TeCS I, 6.9-59% for TeCS II and 5.6-65% for PeCS, respectively. In another experiments to estimate the bioconcentration potential of those compounds, concentration ratios were calculated between river water and fish samples collected from the same locations. The ratios were approximately 100, and higher chlorination indicated the tendency of the compounds being accumulated more to aquatic organisms. Further investigation including of toxic effects about TCS and its chlorinated derivatives will be necessary.



**Fig. 2.** Concentrations of TCS and its chlorinated derivatives in river water and effluent from wastewater treatment plant (ng/l; total amount of both dissolved and particles-bound forms).

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